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(54) Title: ALZHEIMER'S DISEASE SECRETASE

(57) Abstract

The present invention provides the enzyme and enzymatic procedures for cleaving the β secretase cleavage site of the APP protein and associated nucleic acids, peptides, vectors, cells and cell isolates and assays.

Alzheimer's Disease Secretase

FIELD OF THE INVENTION

The present invention related to the field of Alzheimer's Disease, APP, amyloid beta peptide, and human aspartyl proteases as well as a method for the identification of agents that modulate the activity of these polypeptides.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) causes progressive dementia with consequent formation of amyloid plaques, neurofibrillary tangles, gliosis and neuronal loss. The disease occurs in both genetic and sporadic forms whose clinical course and pathological features are quite similar. Three genes have been discovered to date which when mutated cause an autosomal dominant form of Alzheimer's disease. These encode the amyloid protein precursor (APP) and two related proteins, presenilin-1 (PS1) and presenilin-2 (PS2), which as their names suggest are both structurally and functionally related. Mutations in any of the three enhance proteolytic processing of APP via an intracellular pathway that produces amyloid beta peptide or the Aβ peptide (or sometimes here as Abeta), a 40-42 amino acid long peptide that is the primary component of amyloid plaque in AD. Dysregulation of intracellular pathways for proteolytic processing may be central to the pathophysiology of AD. In the case of plaque formation, mutations in APP, PS1 or PS2 consistently alter the proteolytic processing of APP so as to enhance formation of Aβ 1-42, a form of the Aβ peptide which seems to be particularly amyloidogenic, and thus very important in AD. Different forms of APP range in size from 695-770 amino acids, localize to the cell surface, and have a single C-terminal transmembrane domain. The Abeta peptide is derived from a region of APP adjacent to and containing a portion of the transmembrane domain. Normally, processing of APP at the α-secretase site cleaves the midregion of the Aβ sequence adjacent to the membrane and releases the soluble, extracellular domain of APP from the cell surface. This α-secretase APP processing, creates soluble APP- α, and it is normal and not thought to contribute to AD.

Pathological processing of APP at the β- and γ-secretase sites produces a very different result than processing at the α site. Sequential processing at the β- and γ-secretase sites releases the Aβ peptide, a peptide possibly very important in AD pathogenesis. Processing at the β- and γ-secretase sites can occur in both the endoplasmic reticulum (in neurons) and in the endosomal/lysosomal pathway after reinternalization of cell surface

polynucleotide of claim 4 where the two sets of nucleic acids are separated by nucleic acids that code for about 150 to 190, amino acid (positions). The nucleic acid polynucleotide of claim 9 where the two sets of nucleotides are separated by nucleic acids that code for about 190 amino acids (positions). The nucleic acid polynucleotide of claim 10 where the two sets of nucleotides are separated by the same nucleic acid sequences that separate the same set of special nucleotides in SEQ. ID. NO. 1. Claims 1-11 where the first nucleic acid of the first special set of amino acids, that is, the first special nucleic acid, is operably linked to any codon where the nuclic acids of that codon codes for any peptide comprising from 1 to 10,000 amino acid (positions). The nucleic acid polynucleotide of claims 1-12 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any any reporter proteins or proteins which facilitate purification.The nucleic acid polynucleotide of claims 1-13 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin. Claims 1-14 where the last nucleic acid of the second set of special amino acids, that is, the last special nucleic acid, is operably linked to nucleic acid polymers that code for any peptide comprising any amino acids from 1 to 10,000 amino acids. Claims 1-15 where the last special nucleic acid is operably linked to any codon linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any reporter proteins or proteins which facilitate purification. The nucleic acid polynucleotide of claims 1-16 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for DTG, where the first nucleic acid of the first special set of nucleic acids is, the first special nucleic acid, and where the second set of nucleic acids code for either DSG or DTG, where the last nucleic acid of the second set of special nucleic acids is the last special nucleic acid, where the first special nucleic acid is operably linked to nucleic

to 163 codons. The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 142 codons. The nucleic acid polynucleotide of claim 32 where the polynucleotide comprises a sequence that is at least 95% identical to SEQ. ID. (Example 9 or 10). The nucleic acid polynucleotide of claim 33, where the complete polynucleotide comprises SEQ. ID. (Example 9 or 10). The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 163 codons. The nucleic acid polynucleotide of claim 35 where the polynucleotide comprises a sequence that is at least 95% identical to SEQ. ID. (Example 9 or 10). The nucleic acid polynucleotide of claim 36, where the complete polynucleotide comprises SEQ. ID. (Example 9 or 10). The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 170 codons. Claims 1-38 where the second set of special nucleid acids code for the peptide DSG, and optionally the first set of nucleic acid polynucleotide is operably linked to a peptide purification tag. Claims 1-39 where the nucleic acid polynucleotide is operably linked to a peptide purification tag which is six histidine. Claims 1-40 where the first set of special nucleic acids are on one polynucleotide and the second set of special nucleic acids are on a second polynucleotide, where both first and second polynucleotides have at lease 50 codons. Claims 1-40 where the first set of special nucleic acids are on one polynucleotide and the second set of special nucleic acids are on a second polynucleotide, where both first and second polynucleotides have at lease 50 codons where both said polynucleotides are in the same solution. A vector which contains a polynucleotide described in claims 1-42. A cell or cell line which contans a polynucleotide described in claims 1-42.

Any isolated or purified peptide or protein comprising an amino acid polymer that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid position can be any amino acid, where the first set of special amino acids consists of the peptide DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, where the second set of amino acids is selected from the peptide comprising either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, with the proviso that the proteases disclosed in SEQ ID NO. 2 and SEQ. ID NO. 6 are not included. The amino acid polypeptide of claim 45 where the two sets of amino acids are

separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino acid, where the first set of special amino acids consists of the amino acids DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids is either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, where the first special amino acid is operably linked to amino acids that code for any number of amino acids from zero to 81 amino acid positions where in each position it may be any amino acid. The amino acid polypeptide of claim 62, where the first special amino acid is operably linked to a peptide from about 64 to 77 amino acids positions where each amino acid position may be any amino acid. The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to a peptide of 71 amino acids. The amino acid polypeptide of claim 64, where the first special amino acid is operably linked to 71 amino acids and the first of those 71 amino acids is the amino acid T. The amino acid polypeptide of claim 65, where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. (Example 11). The amino acid polypeptide of claim 66, where the complete polypeptide comprises SEQ. ID. (Example 11). The amino acid polypeptide of claim 62, where the first special amino acid is operably linked to any number of from 40 to 54 amino acids (positions) where each amino acid position may be any amino acid. The amino acid polypeptide of claim 68, where the first special amino acid is operably linked to amino acids that code for a peptide of 47 amino acids. The amino acid polypeptide of claim 69, where the first special amino acid is operably linked to a 47 amino acid peptide where the first those 47 amino acids is the amino acid E. The amino acid polypeptide of claim 70, where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. (Example 10). The amino acid polypeptide where the polypeptide comprises Example 10).

Any isolated or purified amino acid polypeptide that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino acid, where the first set of special amino acids consists of the amino acids that code for DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids are either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, which is operably linked to any number of amino acids from 50 to 170 amino acids, which may be any amino

DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids is either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, where the first special amino acid is operably linked to amino acids that code for any number of amino acids from zero to 81 amino acid positions where in each position it may be any amino acid.

The amino acid polypeptide of claim 62, where the first special amino acid is operably linked to a peptide from about 30 to 77 amino acids positions where each amino acid position may be any amino acid. The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to a peptide of 35, 47, 71, or 77 amino acids.

The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to the same corresponding peptides from SEQ. ID. NO. 3 that are 35, 47, 71, or 77 peptides in length, beginning counting with the amino acids on the first special sequence, DTG, towards the N-terminal of SEQ. ID. NO. 3.

The amino acid polypeptide of claim 65, where the polypeptide comprises a sequence that is at least 95% identical to the same corresponding amino acids in SEQ. ID. NO. 4, that is, identical to that portion of the sequences in SEQ.ID. NO. 4, including all the sequences from both the first and or the second special nucleic acids, toward the N-terminal, through and including 71, 47, 35 amino acids before the first special amino acids. (Examples 10 and 11).

The amino acid polypeptide of claim 65, where the complete polypeptide comprises the peptide of 71 amino acids, where the first of the amino acid is T and the second is Q. The nucleic acid polynucleotide of claim 21, where the polynucleotide comprises a sequence that is at least 95% identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 71 amino acids, see Example 10, beginning from the DTG site and including the nucleotides from that code for 71 amino acids).

The nucleic acid polynucleotide of claim 22, where the complete polynucleotide comprises identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 71

Asp2(a), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID No. 4. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp2(b), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID No. 5. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a) or (b) of claim 92. A vector comprising the nucleic acid molecule of claim 96. The vector of claim 97, wherein said nucleic acid molecule is operably linked to a promoter for the expression of a Hu-Asp polypeptide. The vector of claim 98, wherein said Hu-Asp polypeptide is Hu-Asp1. The vector of claim 98, wherein said Hu-Asp polypeptide is Hu-Asp2(a). The vector of claim 98, wherein said Hu-Asp polypeptide is Hu-Asp2(b). A host cell comprising the vector of claim 98. A method of obtaining a Hu-Asp polypeptide comprising culturing the host cell of claim 102 and isolating said Hu-Asp polypeptide. An isolated Hu-Asp1 polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID No. 2. An isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID No. 4. An isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID No. 8. An isolated antibody that binds specifically to the Hu-Asp polypeptide of any of claims 104-107.

Here we disclose numerous methods to assay the enzyme.

A method to identify a cell that can be used to screen for inhibitors of β secretase activity comprising:

(a) identifying a cell that expresses a protease capable of cleaving APP at the β secretase site, comprising:

i) collect the cells or the supernantent from the cells to be identified

ii) measure the production of a critical peptide, where the critical peptide is selected from the group consisting of either the APP C-terminal peptide or soluble APP,

iii) select the cells which produce the critical peptide.

The method of claim 108 where the cells are collected and the critical peptide is the APP C-terminal peptide created as a result of the β secretase cleavage. The method of claim 108 where the supernantent is collected and the critical peptide is soluble APP where the soluble APP has a C-terminal created by β secretase cleavage. The method of claim 108

a) culturing cells in a culture medium under conditions in which the enzyme causes processing of APP and release of amyloid beta-peptide into the medium and causes the accumulation of CTF99 fragments of APP in cell lysates,

b) exposing the cultured cells to a test compound; and specifically determining whether the test compound inhibits the function of the enzyme by measuring the amount of amyloid beta-peptide released into the medium and or the amount of CTF99 fragments of APP in cell lysates;

c) identifying test compounds diminishing the amount of soluble amyloid beta peptide present in the culture medium and diminution of CTF99 fragments of APP in cell lysates as Asp2 inhibitors.

The method of claim 135 wherein the cultured cells are a human, rodent or insect cell line. The method of claim 136 wherein the human or rodent cell line exhibits β secretase activity in which processing of APP occurs with release of amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates. A method as in claim 137 wherein the human or rodent cell line treated with the antisense oligomers directed against the enzyme that exhibits β secretase activity, reduces release of soluble amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates. A method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising:

a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent;

whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide..The nucleic acids, peptides, proteins, vectors, cells and cell lines, and assays described herein.

The present invention provides isolated nucleic acid molecules comprising a polynucleotide that codes for a polypeptide selected from the group consisting of human aspartyl proteases. In particular, human aspartyl protease 1 (Hu-Asp1) and two alternative splice variants of human aspartyl protease 2 (Hu-Asp2), designated herein as Hu-Asp2(a) and

The invention also provides a method for the identification of an agent that modulates the activity of any of Hu-Asp-1, Hu-Asp2(a), and Hu-Asp2(b). The inventions describes methods to test such agents in cell-free assays to which Hu-Asp2 polypeptide is added, as well as methods to test such agents in human or other mammalian cells in which Hu-Asp2 is present.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

Sequence ID No. 1—Human Asp-1, nucleotide sequence
Sequence ID No. 2—Human Asp-1, predicted amino acid sequence
Sequence ID No. 3—Human Asp-2(a), nucleotide sequence
Sequence ID No. 4—Human Asp-2(a), predicted amino acid sequence
Sequence ID No. 5—Human Asp-2(b), nucleotide sequence
Sequence ID No. 6—Human Asp-2(b), predicted amino acid sequence
Sequence ID No. 7—Murine Asp-2(a), nucleotide sequence
Sequence ID No. 8—Murine Asp-2(a), predicted amino acid sequence
Sequence ID No. 9—Human APP695, nucleotide sequence
Sequence ID No.10—Human APP695, predicted amino acid sequence
Sequence ID No.11—Human APP695-Sw, nucleotide sequence
Sequence ID No.12—Human APP695-Sw. predicted amino acid sequence
Sequence ID No.13—Human APP695-VF, nucleotide sequence
Sequence ID No.14—Human APP695-VF, predicted amino acid sequence
Sequence ID No.15—Human APP695-KK, nucleotide sequence
Sequence ID No.16—Human APP695-KK, predicted amino acid sequence
Sequence ID No.17—Human APP695-Sw-KK, nucleotide sequence
Sequence ID No.18—Human APP695-Sw-KK, predicted amino acid sequence
Sequence ID No.19—Human APP695-VF-KK, nucleotide sequence
Sequence ID No.20—Human APP695-VF-KK, predicted amino acid sequence
Sequence ID No.21—T7-Human-pro-Asp-2(a)ΔTM, nucleotide sequence
Sequence ID No.22—T7-Human-pro-Asp-2(a)ΔTM, amino acid sequence
Sequence ID No.23—T7-Caspase-Human-pro-Asp-2(a)ΔTM, nucleotide sequence
Sequence ID No.24—T7-Caspase-Human-pro-Asp-2(a)ΔTM, amino acid sequence
Sequence ID No.25—Human-pro-Asp-2(a)ΔTM (low GC), nucleotide sequence
Sequence ID No.26—Human-pro-Asp-2(a)ΔTM, (low GC), amino acid sequence
Sequence ID No.27—T7-Caspase-Caspase 8 cleavage-Human-pro-Asp-2(a)ΔTM, nucleotide sequence
Sequence ID No.28—T7-Caspase-Caspase 8 cleavage-Human-pro-Asp-2(a)ΔTM, amino acid sequence
Sequence ID No.29—Human Asp-2(a)ΔTM, nucleotide sequence
Sequence ID No.30—Human Asp-2(a)ΔTM, amino acid sequence
Sequence ID No.31—Human Asp-2(a)ΔTM$(His)_6$, nucleotide sequence
Sequence ID No.32—Human Asp-2(a)ΔTM$(His)_6$, amino acid sequence
Sequence ID No.s 33-46 are described below in the Detailed Description of the Invention.

BRIEF DESCRIPTION OF THE FIGURES

secretase cleavage site to 39, 40, 41, 42 and 43 amino acids. β amyloid peptide also means sequences 1-6, SEQ. ID. NO. 1-6 of US 5,750,349, issued 12 May 1998 (incorporated into this document by reference). A β-secretase cleavage fragment disclosed here is called CTF-99, which extends from β-secretase cleavage site to the carboxy terminus of APP.

When an isoform of APP is discussed then what is meant is any APP polypeptide, including APP variants (including mutations), and APP fragments that exists in humans such as those desribed in US 5,766,846, col 7, lines 45-67, incorporated into this document by reference and see below.

The term "β-amyloid precursor protein" (APP) as used herein is defined as a polypeptide that is encoded by a gene of the same name localized in humans on the long arm of chromosome 21 and that includes "βAP – here "β-amyloid protein" see above, within its carboxyl third. APP is a glycosylated, single-membrane spanning protein expressed in a wide variety of cells in many mammaliam tissues. Examples of specific isotypes of APP which are currently known to exist in humans are the 695-amino acid polypeptide described by Kang et. al. (1987) Nature 325:733-736 which is designated as the "normal" APP; the 751-amino acid polypeptide described by Ponte et al. (1988) Nature 331:525-527 (1988) and Tanzi et al. (1988) Nature 331:528-530; and the 770-amino acid polypeptide described by Kitaguchi et. al. (1988) Nature 331:530-532. Examples of specific variants of APP include point mutation which can differ in both position and phenotype (for review of known variant mutation see Hardy (1992) Nature Genet. 1:233-234). All references cited here incorporated by reference. The term "APP fragments" as used herein refers to fragments of APP other than those which consist solely of βAP or βAP fragments. That is, APP fragments will include amino acid sequences of APP in addition to those which form intact 3AP or a fragment of βAP..

When the term "any amino acid" is used, the amino acids referred to are to be selected from the following, three letter and single letter abbreviations - which may also be used, are provided as follows:

Alanine, Ala, A; Arginine, Arg, R; Asparagine, Asn, N; Aspartic acid, Asp, D; Cystein, Cys, C; Glutamine, Gln, Q; lu;E-Glutamic Acid, Glu, E; Glycine, Gly, G; Histidine, His, H; Isoleucine, Ile, I; Leucine, Leu, L; Lysine, Lys, K; Methionine, Met, M; Phenylalanine, Phe, F; Proline, Pro, P; Serine, Ser, S; Threonine, Thr, T; Tryptophan, Trp, W; Tyrosine, Tyr, Y; Valine, Val, V; Aspartic acid or Asparagine, Asx, B; Glutamic acid or Glutamine, Glx, Z; Any amino acid, Xaa, X..

The present invention involves the molecular definition of several novel human aspartyl proteases and one of these, referred to as Hu-Asp-2(a) and Hu-Asp2(b), has been characterized in detail. Previous forms of asp1 and asp 2 have been disclosed, see EP 0848062 A2 and EP 0855444A2, inventors David Powel et. al., assigned to Smith Kline Beecham Corp. (incorporated by reference). Herein are disclosed old and new forms of Hu-Asp 2. For the first time they are expressed in active form, their substrates are disclosed, and their specificity is disclosed. Prior to this disclosure cell or cell extracts were required to cleave the β-secretase site, now purified protein can be used in assays, also described here. Based on the results of (1) antisense knock out experiments, (2) transient transfection knock in experiments, and (3) biochemical experiments using purified recombinant Hu-Asp-2, we demonstrate that Hu-Asp-2 is the β-secretase involved in the processing of APP. Although the nucleotide and predicted amino acid sequence of Hu-Asp-2(a) has been reported, see above, see EP 0848062 A2 and EP 0855444A2, no functional characterization of the enzyme was disclosed. Here the authors characterize the Hu-Asp-2 enzyme and are able to explain why it is a critical and essential enzyme required in the formation of $A\beta_{1-42}$, peptide and possible a critical step in the development of AD.

In another embodiment the present invention also describes a novel splice variant of Hu-Asp2, referred to as Hu-Asp-2(b), that has never before been disclosed.

In another embodiment, the invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a polypeptide selected from the group consisting of human aspartyl protease 1 (Hu-Asp1) and two alternative splice variants of human aspartyl protease 2 (Hu-Asp2), designated herein as Hu-Asp2(a) and Hu-Asp2(b). As used herein, all references to "Hu-Asp2" should be understood to refer to both Hu-Asp2(a) and Hu-Asp2(b). Hu-Asp1 is expressed most abundantly in pancreas and prostate tissues, while Hu-Asp2(a) and Hu-Asp2(b) are expressed most abundantly in pancreas and brain tissues. The invention also provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as fragments thereof which exhibit aspartyl protease activity.

The predicted amino acid sequences of Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) share significant homology with previously identified mammalian aspartyl proteases such as pepsinogen A, pepsinogen B, cathepsin D, cathepsin E, and renin. P.B.Szecs, *Scand. J. Clin. Lab. Invest.* 52:(Suppl. 210 5-22 (1992)). These enzymes are characterized by the presence of a duplicated DTG/DSG sequence motif. The Hu-Asp1 and HuAsp2 polypeptides disclosed

Also provided herein are purified Hu-Asp polypeptides, both recombinant and non-recombinant. Most importantly, methods to produce Hu-Asp2 polypeptides in active form are provided. These include production of Hu-Asp2 polypeptides and variants thereof in bacterial cells, insect cells, and mammalian cells, also in forms that allow secretion of the Hu-Asp2 polypeptide from bacterial, insect or mammalian cells into the culture medium, also methods to produce variants of Hu-Asp2 polypeptide incorporating amino acid tags that facilitate subsequent purification. In a preferred embodiment of the invention the Hu-Asp2 polypeptide is converted to a proteolytically active form either in transformed cells or after purification and cleavage by a second protease in a cell-free system, such active forms of the Hu-Asp2 polypeptide beginning with the N-terminal sequence TQHGIR or ETDEEP. Variants and derivatives, including fragments, of Hu-Asp proteins having the native amino acid sequences given in SEQ ID Nos: 2, 4, and 6 that retain any of the biological activities of Hu-Asp are also within the scope of the present invention. Of course, one of ordinary skill in the art will readily be able to determine whether a variant, derivative, or fragment of a Hu-Asp protein displays Hu-Asp activity by subjecting the variant, derivative, or fragment to a standard aspartyl protease assay. Fragments of Hu-Asp within the scope of this invention include those that contain the active site domain containing the amino acid sequence DTG, fragments that contain the active site domain amino acid sequence DSG, fragments containing both the DTG and DSG active site sequences, fragments in which the spacing of the DTG and DSG active site sequences has been lengthened, fragments in which the spacing has been shortened. Also within the scope of the invention are fragments of Hu-Asp in which the transmembrane domain has been removed to allow production of Hu-Asp2 in a soluble form. In another embodiment of the invention, the two halves of Hu-Asp2, each containing a single active site DTG or DSG sequence can be produced independently as recombinant polypeptides, then combined in solution where they reconstitute an active protease.

Hu-Asp variants may be obtained by mutation of native Hu-Asp-encoding nucleotide sequences, for example. A Hu-Asp variant, as referred to herein, is a polypeptide substantially homologous to a native Hu-Asp polypeptide but which has an amino acid sequence different from that of native Hu-Asp because of one or more deletions, insertions, or substitutions in the amino acid sequence. The variant amino acid or nucleotide sequence is preferably at least about 80% identical, more preferably at least about 90% identical, and most preferably at least about 95% identical, to a native Hu-Asp sequence. Thus, a variant nucleotide sequence which contains, for example, 5 point mutations for every one hundred

By stringent hybridization conditions is intended overnight incubation at about 42°C for about 2.5 hours in 6 X SSC/0.1% SDS, followed by washing of the filters in 1.0 X SSC at 65°C, 0.1% SDS.

Fragments of the Hu-Asp-encoding nucleic acid molecules described herein, as well as polynucleotides capable of hybridizing to such nucleic acid molecules may be used as a probe or as primers in a polymerase chain reaction (PCR). Such probes may be used, *e.g.*, to detect the presence of Hu-Asp nucleic acids in *in vitro* assays, as well as in Southern and northern blots. Cell types expressing Hu-Asp may also be identified by the use of such probes. Such procedures are well known, and the skilled artisan will be able to choose a probe of a length suitable to the particular application. For PCR, 5' and 3' primers corresponding to the termini of a desired Hu-Asp nucleic acid molecule are employed to isolate and amplify that sequence using conventional techniques.

Other useful fragments of the Hu-Asp nucleic acid molecules are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence capable of binding to a target Hu-Asp mRNA (using a sense strand), or Hu-Asp DNA (using an antisense strand) sequence. In a preferred embodiment of the invention these Hu-Asp antisense oligonucleotides reduce Hu-Asp mRNA and consequent production of Hu-Asp polypeptides.

In another aspect, the invention includes Hu-Asp polypeptides with or without associated native pattern glycosylation. Both Hu-Asp1 and Hu-Asp2 have canonical acceptor sites for Asn-linked sugars, with Hu-Asp1 having two of such sites, and Hu-Asp2 having four. Hu-Asp expressed in yeast or mammalian expression systems (discussed below) may be similar to or significantly different from a native Hu-Asp polypeptide in molecular weight and glycosylation pattern. Expression of Hu-Asp in bacterial expression systems will provide non-glycosylated Hu-Asp.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Hu-Asp polypeptides may be recovered and purified from tissues, cultured cells, or recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, lectin chromatography, and high performance liquid chromatography (HPLC). In a preferred embodiment, an amino acid tag is added to the Hu-Asp polypeptide using genetic engineering techniques that are well known to practioners of the art which include addition of six histidine amino acid residues to allow

extracellular secretion of the Hu-Asp polypeptide. Preferably, the signal sequence will be cleaved from the Hu-Asp polypeptide upon secretion of Hu-Asp from the cell. Non-limiting examples of signal sequences that can be used in practicing the invention include the yeast I-factor and the honeybee melatin leader in sf9 insect cells.

In a preferred embodiment, the Hu-Asp polypeptide will be a fusion protein which includes a heterologous region used to facilitate purification of the polypeptide. Many of the available peptides used for such a function allow selective binding of the fusion protein to a binding partner. For example, the Hu-Asp polypeptide may be modified to comprise a peptide to form a fusion protein which specifically binds to a binding partner, or peptide tag. Non-limiting examples of such peptide tags include the 6-His tag, thioredoxin tag, hemaglutinin tag, GST tag, and OmpA signal sequence tag. As will be understood by one of skill in the art, the binding partner which recognizes and binds to the peptide may be any molecule or compound including metal ions *(e.g.,* metal affinity columns), antibodies, or fragments thereof, and any protein or peptide which binds the peptide, such as the FLAG tag.

Suitable host cells for expression of Hu-Asp polypeptides includes prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the expression of Hu-Asp include bacteria of the genera *Escherichia, Bacillus, and Salmonella,* as well as members of the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.* For expression in, *e.g., E. coli,* a Hu-Asp polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in a prokaryotic host. The N-terminal Met may optionally then be cleaved from the expressed Hu-Asp polypeptide. Other N-terminal amino acid residues can be added to the Hu-Asp polypeptide to facilitate expression in Escherichia coli including but not limited to the T7 leader sequence, the T7-caspase 8 leader sequence, as well as others leaders including tags for purification such as the 6-His tag (Example 9). Hu-Asp polypeptides expressed in E. coli may be shortened by removal of the cytoplasmic tail, the transmembrane domain, or the membrane proximal region. Hu-Asp polypeptides expressed in E. coli may be obtained in either a soluble form or as an insoluble form which may or may not be present as an inclusion body. The insoluble polypeptide may be rendered soluble by guanidine HCl, urea or other protein denaturants, then refolded into a soluble form before or after purification by dilution or dialysis into a suitable aqueous buffer. If the inactive proform of the Hu-Asp was produced using recombinant methods, it may be rendered active by cleaving off the prosegment with a second suitable protease such as human immunodeficiency virus protease.

from viral genomes. Commonly used promoter sequences and enhancer sequences which may be used in the present invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40). Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol. 3*:280 (1983)); Cosman *et al.* (*Mol. Immunol. 23*:935 (1986)); Cosman *et al.* (*Nature 312*:768 (1984)); EP-A-0367566; and WO 91/18982.

The polypeptides of the present invention may also be used to raise polyclonal and monoclonal antibodies, which are useful in diagnostic assays for detecting Hu-Asp polypeptide expression. Such antibodies may be prepared by conventional techniques. See, for example, *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet *et al.* (eds.), Plenum Press, New York (1980). Synthetic peptides comprising portions of Hu-Asp containing 5 to 20 amino acids may also be used for the production of polyclonal or monoclonal antibodies after linkage to a suitable carrier protein including but not limited to keyhole limpet hemacyanin (KLH), chicken ovalbumin, or bovine serum albumin using various cross-linking reagents including carbodimides, glutaraldehyde, or if the peptide contains a cysteine, N-methylmaleimide. A preferred peptide for immunization when conjugated to KLH contains the C-terminus of Hu_Asp1 or Hu-Asp2 comprising QRRPRDPEVVNDESSLVRHRWK or LRQQHDDFADDISLLK, respectively.

The Hu-Asp nucleic acid molecules of the present invention are also valuable for chromosome identification, as they can hybridize with a specific location on a human chromosome. Hu-Asp1 has been localized to chromosome 21, while Hu-Asp2 has been localized to chromosome 11q23.3-24.1. There is a current need for identifying particular sites on the chromosome, as few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. The relationship between genes and diseases that have been mapped to the same chromosomal region can then be identified through linkage analysis, wherein the coinheritance of physically adjacent genes is determined. Whether a gene appearing to be related to a particular disease is in fact the cause of the disease can then be determined by comparing the nucleic acid sequence between affected and unaffected individuals.

(a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

(b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent;

whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide. Such tests can be performed with Hu-Asp polypeptide in a cell free system and with cultured cells that express Hu-Asp as well as variants or isoforms thereof.

In another embodiment, the invention relates to a novel cell line (HEK125.3 cells) for measuring processing of amyloid β peptide (Aβ) from the amyloid protein precursor (APP). The cells are stable transformants of human embryonic kidney 293 cells (HEK293) with a bicistronic vector derived from pIRES-EGFP (Clontech) containing a modified human APP cDNA, an internal ribosome entry site and an enhanced green fluorescent protein (EGFP) cDNA in the second cistron. The APP cDNA was modified by adding two lysine codons to the carboxyl terminus of the APP coding sequence. This increases processing of Aβ peptide from human APP by 2-4 fold. This level of Aβ peptide processing is 60 fold higher than is seen in nontransformed HEK293 cells. HEK125.3 cells will be useful for assays of compounds that inhibit Aβ peptide processing. This invention also includes addition of two lysine residues to the C-terminus of other APP isoforms including the 751 and 770 amino acid isoforms, to isoforms of APP having mutations found in human AD including the Swedish KM→NL and V717→F mutations, to C-terminal fragments of APP, such as those beginning with the β-secretase cleavage site, to C-terminal fragments of APP containing the β-secretase cleavage site which have been operably linked to an N-terminal signal peptide for membrane insertion and secretion, and to C-terminal fragments of APP which have been operably linked to an N-terminal signal peptide for membrane insertion and secretion and a reporter sequence including but not limited to green fluorescent protein or alkaline phosphatase, such that β-secretase cleavage releases the reporter protein from the surface of cells expressing the polypeptide.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

specifically for this purpose. The following AWK script for locating proteins containing two DTG or DSG motifs was used for the search, which was repeated four times to recover all pairwise combinations of the aspartyl motif.

```
BEGIN{RS=">"}            /* defines ">" as record separator for FASTA format */
{
pos = index($0,"DTG")    /*finds "DTG" in record*/
if (pos>0) {
        rest = substr($0,pos+3)      /*get rest of record after first DTG*/
        pos2 = index(rest,"DTG")     /*find second DTG*/
        if (pos2>0) printf ("%s%s\n",">",$0)}      /*report hits*/
}
}
```

The AWK script shown above was used to search Wormpep12, which was downloaded from ftp.sanger.ac.uk/pub/databases/wormpep, for sequence entries containing at least two DTG or DSG motifs. Using AWK limited each record to 3000 characters or less. Thus, 35 or so larger records were eliminated manually from Wormpep12 as in any case these were unlikely to encode aspartyl proteases.

Results and Discussion:

The Wormpep 12 database contains 12,178 entries, although some of these (<10%) represent alternatively spliced transcripts from the same gene. Estimates of the number of genes encoded in the *C. elegans* genome is on the order of 13,000 genes, so Wormpep12 may be estimated to cover greater than 90% of the *C. elegans* genome.

Eukaryotic aspartyl proteases contain a two-domain structure, probably arising from ancestral gene duplication. Each domain contains the active site motif D(S/T)G located from 20-25 amino acid residues into each domain. The retroviral (e.g., HIV protease) or retrotransposon proteases are homodimers of subunits which are homologous to a single eukaryotic aspartyl protease domain. An AWK script was used to search the Wormpep12 database for proteins in which the D(S/T)G motif occurred at least twice. This identified >60 proteins with two DTG or DSG motifs. Visual inspection was used to select proteins in which the position of the aspartyl domains was suggestive of a two-domain structure meeting the criteria described above.

In addition, the PROSITE eukaryotic and viral aspartyl protease active site pattern PS00141 was used to search Wormpep12 for candidate aspartyl proteases. (Bairoch A., Bucher P., Hofmann K., The PROSITE database: its status in 1997, *Nucleic Acids Res.* *24*:217-221(1997)). This generated an overlapping set of Wormpep12 sequences. Of these,

Example 2: Identification of Novel Human Aspartyl Proteases Using Database Mining by Genome Bridging

Materials and Methods:

Computer-assisted analysis of EST databases, cDNA , and predicted polypeptide sequences:

Exhaustive homology searches of EST databases with the CEASP1, F21F8.3, F21F8.4, and F21F8.7 sequences failed to reveal any novel mammalian homologues. TBLASTN searches with R12H7.2 showed homology to cathepsin D, cathepsin E, pepsinogen A, pepsinogen C and renin, particularly around the DTG motif within the active site, but also failed to identify any additional novel mammalian aspartyl proteases. This indicates that the *C. elegans* genome probably contains only a single lysosomal aspartyl protease which in mammals is represented by a gene family that arose through duplication and consequent modification of an ancestral gene.

TBLASTN searches with T18H9.2, the remaining *C. elegans* sequence, identified several ESTs which assembled into a contig encoding a novel human aspartyl protease (Hu-ASP1). As is described above in Example 1, BLASTX search with the Hu-ASP1 contig against SWISS-PROT revealed that the active site motifs in the sequence aligned with the active sites of other aspartyl proteases. Exhaustive, repetitive rounds of BLASTN searches against LifeSeq, LifeSeqFL, and the public EST collections identified 102 EST from multiple cDNA libraries that assembled into a single contig. The 51 sequences in this contig found in public EST collections also have been assembled into a single contig (THC213329) by The Institute for Genome Research (TIGR). The TIGR annotation indicates that they failed to find any hits in the database for the contig. Note that the TIGR contig is the reverse complement of the LifeSeq contig that we assembled. BLASTN search of Hu-ASP1 against the rat and mouse EST sequences in ZooSeq revealed one homologous EST in each database (Incyte clone 700311523 and IMAGE clone 313341, GenBank accession number W10530, respectively).

TBLASTN searches with the assembled DNA sequence for Hu-ASP1 against both LifeSeqFL and the public EST databases identified a second, related human sequence (Hu-Asp2) represented by a single EST (2696295). Translation of this partial cDNA sequence reveals a single DTG motif which has homology to the active site motif of a bovine aspartyl protease, NM1.

(2696295CE1) with approximately 60% shared identity was found in the LifeSeq FL database. Similar queries of either gb105PubEST or the family of human databases available from TIGR did not identify similar EST clones. cDNA clone 2696295, identified by single pass sequence analysis from a human uterus cDNA library, was obtained from Incyte and completely sequence on both strands. This clone contained an incomplete 1266 bp open-reading frame that encoded a 422 amino acid polypeptide but lacked an initiator ATG on the 5' end. Inspection of the predicted sequence revealed the presence of the duplicated aspartyl protease active site motif DTG/DSG, separated by 194 amino acid residues. Subsequent queries of later releases of the LifeSeq EST database identified an additional ESTs, sequenced from a human astrocyte cDNA library (4386993), that appeared to contain additional 5' sequence relative to clone 2696295. Clone 4386993 was obtained from Incyte and completely sequenced on both strands. Comparative analysis of clone 4386993 and clone 2696295 confirmed that clone 4386993 extended the open-reading frame by 31 amino acid residues including two in-frame translation initiation codons. Despite the presence of the two in-frame ATGs, no in-frame stop codon was observed upstream of the ATG indicating that the 4386993 may not be full-length. Furthermore, alignment of the sequences of clones 2696295 and 4386993 revealed a 75 base pair insertion in clone 2696295 relative to clone 4386993 that results in the insertion of 25 additional amino acid residues in 2696295. The remainder of the Hu-Asp2 coding sequence was determined by 5' Marathon RACE analysis using a human hippocampus Marathon ready cDNA template (Clonetech). A 3'-antisense oligonucleotide primer specific for the shared 5'-region of clones 2696295 and 4386993 was paired with the 5'-sense primer specific for the Marathon ready cDNA synthetic adaptor in the PCR. Specific PCR products were directly sequenced by cycle sequencing and the resulting sequence assembled with the sequence of clones 2696295 and 4386993 to yield the complete coding sequence of Hu-Asp2(a) (SEQ ID No. 3) and Hu-Asp2(b) (SEQ ID No. 5), respectively.

Several interesting features are present in the primary amino acid sequence of Hu-Asp2(a) (Figure 2 and SEQ ID No. 4) and Hu-Asp-2(b) (Figure 3, SEQ ID No. 6). Both sequences contain a signal peptide (residues 1-21 in SEQ ID No. 4 and SEQ ID No. 6), a pro-segment, and a catalytic domain containing two copies of the aspartyl protease active site motif (DTG/DSG). The spacing between the first and second active site motifs is variable due to the 25 amino acid residue deletion in Hu-Asp-2(b) and consists of 168-*versus*-194 amino acid residues, for Hu-Asp2(b) and Hu-Asp-2(a), respectively. More

screen a murine BAC library by PCR, a single genomic clone was isolated and this cloned was confirmed contain the murine Asp2 gene by DNA sequence analysis. Shotgun DNA sequencing of this Asp2 genomic clone and comparison to the cDNA sequences of both Hu_Asp2 and the partial murine cDNA sequences defined the full-length sequence of murine Asp2 (SEQ ID No. 7). The predicted amino acid sequence of murine Asp2 (SEQ ID No. 8) showed 96.4% shared identity (GCG BestFit algorithm) with 18/501 amino acid residue substitutions compared to the human sequence (Figure 4).

Example 4: Tissue Distribution of Expression of Hu-Asp2 Transcripts:

Materials and Methods:

The tissue distribution of expression of Hu-Asp-2 was determined using multiple tissue Northern blots obtained from Clonetech (Palo Alto, CA). Incyte clone 2696295 in the vector pINCY was digested to completion with *EcoRI/NotI* and the 1.8 kb cDNA insert purified by preparative agarose gel electrophoresis. This fragment was radiolabeled to a specific activity > 1 X 10^9 dpm/µg by random priming in the presence of [α-^{32}P-dATP] (>3000 Ci/mmol, Amersham, Arlington Heights, IL) and Klenow fragment of DNA polymerase I. Nylon filters containing denatured, size fractionated poly A^+ RNAs isolated from different human tissues were hybridized with 2 x 10^6 dpm/ml probe in ExpressHyb buffer (Clonetech, Palo Alto, CA) for 1 hour at 68 °C and washed as recommended by the manufacture. Hybridization signals were visualized by autoradiography using BioMax XR film (Kodak, Rochester, NY) with intensifying screens at -80 °C.

Results and Discussion:

Limited information on the tissue distribution of expression of Hu-Asp-2 transcripts was obtained from database analysis due to the relatively small number of ESTs detected using the methods described above (< 5). In an effort to gain further information on the expression of the Hu-Asp2 gene, Northern analysis was employed to determine both the size(s) and abundance of Hu-Asp2 transcripts. PolyA^+ RNAs isolated from a series of peripheral tissues and brain regions were displayed on a solid support following separation under denaturing conditions and Hu-Asp2 transcripts were visualized by high stringency hybridization to radiolabeled insert from clone 2696295. The 2696295 cDNA probe visualized a constellation of transcripts that migrated with apparent sizes of 3.0kb, 4.4 kb and 8.0 kb with the latter two transcript being the most abundant.

process and release Aβ peptide, levels of endogenous APP processing are low and difficult to detect by EIA. Aβ processing can be increased by expressing in transformed cell lines mutations of APP that enhance Aβ processing. We made the serendipitous observation that addition of two lysine residues to the carboxyl terminus of APP695 increases Aβ processing still further. This allowed us to create a transformed cell line that releases Aβ peptide into the culture medium at the remarkable level of 20,000 pg/ml.

Materials And Methods

Materials:

Human embryonic kidney cell line 293 (HEK293 cells) were obtained internally. The vector pIRES-EGFP was purchased from Clontech. Oligonucleotides for mutation using the polymerase chain reaction (PCR) were purchased from Genosys. A plasmid containing human APP695 (SEQ ID No. 9 [nucleotide] and SEQ ID No. 10 [amino acid]) was obtained from Northwestern University Medical School. This was subcloned into pSK (Stratagene) at the *Not*1 site creating the plasmid pAPP695.

Mutagenesis protocol:

The Swedish mutation (K670N, M671L) was introduced into pAPP695 using the Stratagene Quick Change Mutagenesis Kit to create the plasmid pAPP695NL (SEQ ID No. 11 [nucleotide] and SEQ ID No. 12 [amino acid]). To introduce a di-lysine motif at the C-terminus of APP695, the forward primer #276 5' GACTGACCACTCGACCAGGTTC (SEQ ID No. 47) was used with the "patch" primer #274 5' CGAATTAAATTCCAGCACACTGGCTACTTCTTGTTCTGCATCTCAAAGAAC (SEQ ID No. 48) and the flanking primer #275 CGAATTAAATTCCAGCACACTGGCTA (SEQ ID No. 49) to modify the 3' end of the APP695 cDNA (SEQ ID No. 15 [nucleotide] and SEQ ID No. 16 [amino acid]). This also added a BstX1 restriction site that will be compatible with the BstX1 site in the multiple cloning site of pIRES-EGFP. PCR amplification was performed with a Clontech HF Advantage cDNA PCR kit using the polymerase mix and buffers supplied by the manufacturer. For "patch" PCR, the patch primer was used at 1/20th the molar concentration of the flanking primers. PCR amplification products were purified using a QIAquick PCR purification kit (Qiagen). After digestion with restriction enzymes, products were separated on 0.8% agarose gels and then excised DNA fragments were purified using a QIAquick gel extraction kit (Qiagen).

To reassemble a modified APP695-Sw cDNA, the 5' Not1-Bgl2 fragment of the APP695-Sw cDNA and the 3' Bgl2-BstX1 APP695 cDNA fragment obtained by PCR were

164 (New York State Institute for Basic Research, Staten Island, NY) in a double antibody sandwich ELISA. The capture antibody 6E10 is specific to an epitope present on the N-terminal amino acid residues 1-16 of hAβ. The conjugated detecting antibodies 162 and 164 are specific for hAβ 1-40 and 1-42, respectively. Briefly, a Nunc Maxisorp 96 well immunoplate was coated with 100 µl/well of mAb 6E10 (5µg/ml) diluted in 0.1M carbonate-bicarbonate buffer, pH 9.6 and incubated at 4°C overnight. After washing the plate 3x with 0.01M DPBS (Modified Dulbecco's Phosphate Buffered Saline (0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01 M potassium chloride, pH 7.4) from Pierce, Rockford, Il) containing 0.05% of Tween-20 (DPBST), the plate was blocked for 60 min with 200µl of 10% normal sheep serum (Sigma) in 0.01M DPBS to avoid non-specific binding. Human Aβ 1-40 or 1-42 standards 100µl/well (Bachem, Torrance, CA) diluted, from a 1mg/ml stock solution in DMSO, in culture medium was added after washing the plate, as well as 100µl/well of sample, e.g.conditioned medium of transfected cells. The plate was incubated for 2 hours at room temperature and 4°C overnight. The next day, after washing the plate, 100µl/well biotinylated rabbit antiserum 162 1:400 or 164 1:50 diluted in DPBST + 0.5% BSA was added and incubated at room temperature for 1hr 15 min. Following washes, 100µl/well neutravidin-horseradish peroxidase (Pierce, Rockford, Il) diluted 1:10,000 in DPBST was applied and incubated for 1 hr at room temperature. After the last washes 100µl/well of o-phenylnediamine dihydrochloride (Sigma Chemicals, St. Louis, MO) in 50mM citric acid/100mM sodium phosphate buffer (Sigma Chemicals, St. Louis, MO), pH 5.0, was added as substrate and the color development was monitored at 450nm in a kinetic microplate reader for 20 min. using Soft max Pro software. All standards and samples were run in triplicates. The samples with absorbance values falling within the standard curve were extrapolated from the standard curves using Soft max Pro software and expressed in pg/ml culture medium.

Results:

Addition of two lysine residues to the carboxyl terminus of APP695 greatly increases Aβ processing in HEK293 cells as shown by transient expression (Table 1). Addition of the di-lysine motif to APP695 increases Aβ processing to that seen with the APP695 containing the Swedish mutation. Combining the di-lysine motif with the Swedish mutation further increases processing by an additional 2.8 fold.

Island, NY). The diluted stocks of oligofectin G and antisense oligomer were then mixed at a ratio of 1:1 and incubated at room temperature. After 15 min incubation, the reagent was diluted 10 fold into MEM containing 10% fetal calf serum and 2 ml was added to each well of the 6 well plate after first removing the old medium. After transfection, cells were grown in the continual presence of the oligofectin G/antisense oligomer. To monitor Ab peptide release, 400 µl of conditioned medium was removed periodically from the culture well and replaced with fresh medium beginning 24 hr after transfection. Data reported are from culture supernatants harvested 48 hr after transfection.

Results:

The 16 different antisense oligomers obtained from Sequitur Inc were transfected separately into HEK125.3 cells to determine their affect on Aβ peptide processing. Only antisense oligomers targeted against Asp1 & Asp2 reduced Abeta processing by HEK125.3 cells with those targeted against Asp2 having a greater inhibitory effect. Both Aβ (1-40) and Aβ (1-42) were inhibited by the same degree. In Table 3, percent inhibition is calculated with respect to untransfected cells. Antisense oligomer reagents giving greater than 50% inhibition are marked with an asterisk. Of the reagents tested, 3 of 4 antisense oligomers targeted against ASP1 gave an average 52% inhibition of Aβ 1-40 processing and 47% inhibition of Aβ 1-42 processing. For ASP2, 4 of 4 antisense oligomers gave greater than 50% inhibition with an average inhibition of 62% for Aβ 1-40 processing and 60% for Aβ 1-42 processing.

Table 3. Inhibition of Aβ peptide release from HEK125.3 cells treated with antisense oligomers.

Gene Targeted	Antisense Oligomer	Abeta (1-40)	Abeta (1-42)
Asp1-1	S 644	62%*	56%*
Asp1-2	S 645	41%*	38%*
Asp1-3	S646	52%*	46%*
Asp1-4	S647	6%	25%
Asp2-1	S648	71%*	67%*
Asp2-2	S649	83%*	76%*
Asp2-3	S650	46%*	50%*
Asp2-4	S651	47%*	46%*
Con1-1	S652	13%	18%
Con1-2	S653	35%	30%
Con1-3	S655	9%	18%
Con1-4	S674	29%	18%
Con2-1	S656	12%	18%
Con2-2	S657	16%	19%
Con2-3	S658	8%	35%

Example 8. Demonstration of Hu-Asp2 β- Secretase Activity in Cultured Cells

Several mutations in APP associated with early onset Alzheimer's disease have been shown to alter Aβ peptide processing. These flank the N- and C-terminal cleavage sites that release A□ from APP. These cleavage sites are referred to as the β-secretase and γ-secretase cleavage sites, respectively. Cleavage of APP at the β-secretase site creates a C-terminal fragment of APP containing 99 amino acids of 11,145 daltons molecular weight. The Swedish KM→NL mutation immediately upstream of the β-secretase cleavage site causes a general increase in production of both the 1-40 and 1-42 amino acid forms of A□ peptide. The London VF mutation (V717→F in the APP770 isoform) has little effect on total A□ peptide production, but appears to preferentially increase the percentage of the longer 1-42 amino acid form of A□ peptide by affecting the choice of γ-secretase cleavage site used during APP processing. Thus, we sought to determine if these mutations altered the amount and type of A□ peptide produced by cultured cells cotransfected with a construct directing expression of Hu-Asp2.

Two experiments were performed which demonstrate Hu-Asp2 β-secretase activity in cultured cells. In the first experiment, treatment of HEK125.3 cells with antisense oligomers directed against Hu-Asp2 transcripts as described in Example 7 was found to decrease the amount of the C-terminal fragment of APP created by β-secretase cleavage (CTF99) (Figure 9). This shows that Hu-Asp2 acts directly or indirectly to facilitate β-secretase cleavage. In the second experiment, increased expression of Hu-Asp2 in transfected mouse Neuro2A cells is shown to increase accumulation of the CTF99 β-secretase cleavage fragment (Figure 10). This increase is seen most easily when a mutant APP-KK clone containing a C-terminal di-lysine motif is used for transfection. A further increase is seen when Hu-Asp2 is cotransfected with APP-Sw-KK containing the Swedish mutation KM →NL. The Swedish mutation is known to increase cleavage of APP by the β-secretase.

Human embryonic kidney HEK293 cells and mouse Neuro-2a cells were transfected with expression constructs using the Lipofectamine Plus reagent from Gibco/BRL. Cells were seeded in 24 well tissue culture plates to a density of 70-80% confluence. Four wells per plate were transfected with 2 μg DNA (3:1, APP:cotransfectant), 8μl Plus reagent, and 4μl Lipofectamine in OptiMEM. OptiMEM was added to a total volume of 1 ml, distributed 200 μl per well and incubated 3 hours. Care was taken to hold constant the ratios of the two plasmids used for cotransfection as well as the total amount of DNA used in the transfection. The transfection media was replaced with DMEM, 10%FBS, NaPyruvate, with antibiotic/antimycotic and the cells were incubated under normal conditions (37°, 5% CO_2) for 48 hours. The conditioned media were removed to polypropylene tubes and stored at –80°C until assayed for the content of Aβ1-40 and Aβ1-42 by EIA as described in the preceding examples. Transfection of antisense oligomers into HEK125.3 cells was as described in Example 7.

Preparation of cell extracts, Western blot protocol

Cells were harvested after being transfected with plasmid DNA for about 60 hours. First, cells were transferred to 15-ml conical tube from the plate and centrifuged at 1,500 rpm for 5 min to remove the medium. The cell pellets were washed with PBS for one time. We then lysed the cells with lysis buffer (10 mM HEPES, pH 7.9, 150 mM NaCl, 10% glycerol, 1 mM EGTA, 1 mM EDTA, 0.1 mM sodium vanadate and 1% NP-40). The lysed cell mixtures were centrifuged at 5000 rpm and the supernatant was stored at -20°C as the cell extracts. Equal amounts of extracts from HEK125.3 cells transfected with the Asp2 antisense oligomers and controls were precipitated with antibody 369 that recognizes the C-terminus of APP and then CTF99 was detected in the immunoprecipitate with antibody 6E10. The experiment was repeated using C8, a second precipitating antibody that also recognizes the C-terminus of APP. For Western blot of extracts from mouse Neuro-2a cells cotransfected with Hu-Asp2 and APP-KK, APP-Sw-KK, APP-VF-KK or APP-VF, equal amounts of cell extracts were electrophoresed through 4-10% or 10-20% Tricine gradient gels (NOVEX, San Diego, CA). Full length APP and the CTF99 β-secretase product were detected with antibody 6E10.

Results

Western blot showing reduction of CTF99 production by HEK125.3 cells transfected with antisense oligomers targeting the Hu-Asp2 mRNA. (right) Western blot showing increase in CTF99 production in mouse Neuro-2a cells cotransfected with Hu-Asp2 and APP-KK. A further increase in CTF99 production is seen in cells cotransfected with Hu-Asp2 and APP-Sw-KK.

Table 4. Results of cotransfecting Hu-Asp2 or pcDNA plasmid DNA with various APP constructs containing the V717→F mutation that modifies γ-secretase processing. Cotransfection with Asp2 consistently increases the ratio of Aβ42/total Aβ. Values tabulated are Aβ peptide pg/ml.

	pcDNA Cotransfection			Asp2 Cotransfection		
	Aβ40	Aβ42	Aβ42/Total	Aβ40	Aβ42	Aβ42/Total
APP	192±18	<4	<2%	188±40	8±10	3.9%
APP-VF	118±15	15±19	11.5%	85±7	24±12	22.4%
APP-KK	352±24	21±6	5.5%	1062±101	226±49	17.5%
APP-VF-KK	230±31	88±24	27.7%	491±35	355±36	42%

Example 9. Bacterial expression of human Asp2L

Expression of recombinant Hu_Asp2L in E. coli.

Hu-Asp2L can be expressed in E. coli after addition of N-terminal sequences such as a T7 tag (SEQ ID No. 21 and No. 22) or a T7 tag followed by a caspase 8 leader sequence (SEQ ID No. 23 and No. 24). Alternatively, reduction of the GC content of the 5' sequence by site directed mutagenesis can be used to increase the yield of Hu-Asp2 (SEQ ID No. 25 and No. 26). In addition, Asp2 can be engineered with a proteolytic cleavage site (SEQ ID No. 27 and No. 28). To produce a soluble protein after expression and refolding, deletion of the transmembrane domain and cytoplasmic tail, or deletion of the membrane proximal region, transmembrane domain, and cytoplasmic tail is preferred.

Methods

In order to reduce the GC content of the 5' terminus of asp2, a pair of antiparallel oligos were designed to change degenerate codon bases in 15 amino acid positions from G/C to A/T (SEQ ID Nos. 25 and 26). The new nucleotide sequence at the 5' end of asp2 did not change the encoded amino acid and was chosen to optimize E. Coli expression. The sequence of the sense linker is 5' CGGCATCCGGCTGCCCCTGCGTAGCGGTCTGGGTGGTGCTCCACTGGGTCTGCG TCTGCCCCGGGAGACCGACGAA G 3' (SEQ ID No. 39). The sequence of the antisense linker is : 5' CTTCGTCGGTCTCCCGGGGCAGACGCAGACCCAGTGGAGCACCACCCAGACCG CTACGCAGGGGCAGCCGGATGCCG 3' (SEQ ID No. 40). After annealing the phosphorylated linkers together in 0.1 M NaCl-10 mM Tris, pH 7.4 they were ligated into unique Cla I and Sma I sites in Hu-Asp2 in the vector pTAC. For inducible expression using induction with isopropyl b-D-thiogalactopyranoside (IPTG), bacterial cultures were grown in LB broth in the presence of ampicillin at 100 ug/ml, and induced in log phase growth at an OD600 of 0.6-1.0 with 1 mM IPTG for 4 hour at 37°C. The cell pellet was harvested by centrifugation.

To create a vector in which the leader sequences can be removed by limited proteolysis with caspase 8 such that this liberates a Hu-Asp2 polypeptide beginning with the N-terminal sequence GSFV (SEQ ID Nos. 27 and 28), the following procedure was followed. Two phosphorylated oligonucleotides containing the caspase 8 cleavage site IETD, #571=5' GATCGATGACTATCTCTGACTCTCCGCTGGACTCTGGTATCGAAACCGACG (SEQ ID No. 41) and #572= GATCCGTCGGTTTCGATACCAGAGTCCAGCGGAGAGTCAGAGATAGTCATC (SEQ ID No. 42) were annealed and ligated into pET23a+ that had been opened with BamHI. After transformation into JM109, the purified vector DNA was recovered and orientation of the insert was confirmed by DNA sequence analysis. +, the following oligonucleotides were used for amplification of the selected Hu-Asp2 sequence: #573=5'AAGGATCCTTTGTGGAGATGGTGGACAACCTG, (SEQ ID No. 43) #554=GAAAGCTTTCATGACTCATCTGTCTGTGGAATGTTG (SEQ ID No. 44) which placed BamHI and HindIII sites flanking the 5' and 3' ends of the insert, respectively. The Asp2 sequence was amplified from the full length Asp2 cDNA cloned into pcDNA3.1 using the Advantage-GC cDNA PCR [Clontech] following the manufacturer's supplied

1.5L with the same solution prior to homogenizing for 5 minutes. After centrifuging at the same speed for 30 minutes, this procedure was repeated. The pellet was then resuspended into about 150ml of cold water, pooling the pellets from the six centrifuge tubes used in the GSA rotor. The pellet has homogenized for 5 minutes at 3,000 rpm, volume adjusted to 250ml with cold water, then spun for 30 minutes. Weight of the resultant pellet was 17.75g.

Summary: Lysis of bacterial pellet in KCl solution, followed by centrifugation in a GSA rotor was used to initially prepare the pellet. The same solution was then used an additional three times for resuspension/homogenization. A final water wash/homogenization was then performed to remove excess KCl and EDTA.

Solublization of rHuAsp2L:

A ratio of 9-10ml/gram of pellet was utilized for solubilizing the rHuAsp2L from the pellet previously described. 17.75g of pellet was thawed, and 150ml of 8M guanidine HCl, 5mM βME, 0.1% DEA, was added. 3M Tris was used to titrate the pH to 8.6. The pellet was initially resuspended into the guanidine solution using a 20mm tissue homogenizer probe at 1000 rpm. The mixture was then stirred at 4°C for 1 hour prior to centrifugation at 12,500rpm for 1 hour in GSA rotor. The resultant supernatant was then centrifuged for 30min at 40,000 x g in an SS-34 rotor. The final supernatant was then stored at –20°C, except for 50ml.

Immobilized Nickel Affinity Chromatography of Solubilized rHuAsp2L:

The following solutions were utilized:

A) 6M Guanidine HCl, 0.1M NaP, pH 8.0, 0.01M Tris, 5mM βME, 0.5mM Imidazole
A') 6M Urea, 20mM NaP, pH 6.80, 50mM NaCl
B') 6M Urea, 20mM NaP, pH 6.20, 50mM NaCl, 12mM Imidazole
C') 6M Urea, 20mM NaP, pH 6.80, 50mM NaCl, 300mM Imidazole

Note: Buffers A' and C' were mixed at the appropriate ratios to give intermediate concentrations of Imidazole.

The 50ml of solubilized material was combined with 50ml of buffer A prior to adding to 100-125ml Qiagen Ni-NTA SuperFlow (pre-equilibrated with buffer A) in a 5 x 10cm Bio-Rad econo column. This was shaken gently overnight at 4°C in the cold room.

Chromatography Steps:

1) Drained the resultant flow through.
2) Washed with 50ml buffer A (collecting into flow through fraction)
3) Washed with 250ml buffer A (wash 1)
4) Washed with 250ml buffer A (wash 2)
5) Washed with 250ml buffer A'

Experiment 3:

89ml of the 150-2 eluate was spiked with 1M DTT, 3M Tris, pH 7.4 and DEA to a final concentration of 6mM, 50mM, and 0.1% respectively. This was diluted suddenly (while stirring) with 445ml of (4°C) cold 20mM NaP, pH 6.8, 150mM NaCl. This solution appeared clear, with no apparent precipitation. The solution was removed to RT and stirred for 10 minutes prior to adding MEA to a final concentration of 0.1mM. This was stirred slowly at RT for 1hr. Cystamine and $CuSO_4$ were then added to final concentrations of 1mM and 10μM respectively. The solution was stirred slowly at RT for 10 minutes prior to being moved to the 4°C cold room and shaken slowly overnight, open to the air.

The following day, the solution (still clear, with no apparent precipitation) was centrifuged at 100,000 x g for 1 hour. Supernatants from multiple runs were pooled, and the bulk of the stabilized protein was dialyzed against 20mM NaP, pH 7.4, 150mM NaCl, 20% glycerol. After dialysis, the material was stored at -20°C.

Some (about 10ml) of the protein solution (still in 1M Urea) was saved back for biochemical analyses, and frozen at -20°C for storage.

Example 10. Expression of Hu-Asp2 and Derivatives in Insect Cells

Expression by baculovirus infection—The coding sequence of Hu-Asp2 and several derivatives were engineered for expression in insect cells using the PCR. For the full-length sequence, a 5'-sense oligonucleotide primer that modified the translation initiation site to fit the Kozak consensus sequence was paired with a 3'-antisense primer that contains the natural translation termination codon in the Hu-Asp2 sequence. PCR amplification of the pcDNA3.1(hygro)/Hu-Asp2 template (see Example 12). Two derivatives of Hu-Asp2 that delete the C-terminal transmembrane domain (SEQ ID No. 29 and No. 30) or delete the transmembrane domain and introduce a hexa-histidine tag at the C-terminus (SEQ ID No. 31 and No. 32) were also engineered using the PCR. The same 5'-sense oligonucleotide primer described above was paired with either a 3'-antisense primer that (1) introduced a translation termination codon after codon 453 (SEQ ID No. 3) or (2) incorporated a hexa-histidine tag followed by a translation termination codon in the PCR using pcDNA3.1(hygro)/Hu_Asp-2L as the template. In all cases, the PCR reactions were performed amplified for 15 cycles using *Pwo*I DNA polymerase (Boehringer-Mannheim) as outlined by the supplier. The reaction products were digested to completion with *BamH*I and *Not*I and ligated to *BamH*I and *Not*I digested baculovirus transfer vector pVL1393 (Invitrogen). A portion of the ligations was used to transform competent *E. coli* DH5α cells

chromatography by anion exchange (Tris-HCl, pH 8.0) followed by cation exchange chromatography (Acetate buffer at pH 4.5) using NaCl gradients. The elution profile was monitored by (1) Western blot analysis and (2) by activity assay using the peptide substrate described in Example 12. For the Hu-Asp2ΔTM$(His)_6$, the conditioned medium was dialyzed against Tris buffer (pH 8.0) and purified by sequential chromatography on IMAC resin followed by anion exchange chromatography.

Sequence analysis of the purified Hu-Asp2ΔTM$(His)_6$ protein revealed that the signal peptide had been cleaved [TQHGIRLPLR].

Example 11. Expression of Hu-Asp2 in CHO cells

Heterologous expression of Hu_Asp-2L in CHO-K1 cells—The entire coding sequence of Hu-Asp2 was cloned into the mammalian expression vector pcDNA3.1(+)Hygro (Invitrogen, Carlsbad, CA) which contains the CMV immediate early promotor and bGH polyadenylation signal to drive over expression. The expression plasmid, pcDNA3.1(+)Hygro/Hu-Asp2, was prepared by alkaline lysis and banding in CsCl and completely sequenced on both strands to verify the integrity of the coding sequence.

Wild-type Chinese hamster ovary cells (CHO-K1) were obtained from the ATCC. The cells were maintained in monolayer cultures in α-MEM containing 10% FCS at 37°C in 5% CO_2. Two 100 mm dishes of CHO-K1 cells (60% confluent) were transfected with pcDNA3.1(+)/Hygro alone (mock) or pcDNA3.1(+)Hygro/Hu-Asp2 using the cationic liposome DOTAP as recommended by the supplier. The cells were treated with the plasmid DNA/liposome mixtures for 15 hr and then the medium replaced with growth medium containing 500 Units/ml hygromycin B. In the case of pcDNA3.1(+)Hygro/Hu-Asp2 transfected CHO-K1cells, individual hygromycin B-resistant cells were cloned by limiting dilution. Following clonal expansion of the individual cell lines, expression of Hu-Asp2 protein was accessed by Western blot analysis using a polyclonal rabbit antiserum raised

The β-octylglucoside extract was applied to a Mono Q anion exchange column that was previously equilibrated with 25 mM Tris-HCl (pH 8.0)/50 mM β-octylglucoside. Following sample application, the column was eluted with a linear gradient of increasing NaCl concentration (0-1.0 M over 30 minutes) and individual fractions assayed by Western blot analysis and for β-secretase activity (see below). Fractions containing both Hu_Asp-2L immunoreactivity and β-secretase activity were pooled and dialyzed against 25 mM NaOAc (pH 4.5)/50 mM β-octylglucoside. Following dialysis, precipitated material was removed by centrifugation and the soluble material chromatographed on a MonoS cation exchange column that was previously equilibrated in 25 mM NaOAc (pH 4.5)/ 50 mM β-octylglucoside. The column was eluted using a linear gradient of increasing NaCl concentration (0-1.0 M over 30 minutes) and individual fractions assayed by Western blot analysis and for β-secretase activity. Fractions containing both Hu-Asp2 immunoreactivity and β-secretase activity were combined and determined to be >90% pure by SDS-PAGE/Coomassie Blue staining.

Example 12. Assay of Hu-Asp2 β-secretase activity using peptide substrates

β-secretase assay—β-secretase activity was measured by quantifying the hydrolysis of a synthetic peptide containing the APP Swedish mutation by RP-HPLC with UV detection. Each reaction contained 50 mM Na-MES (pH 5.5), 1% β-octylglucoside, peptide substrate (SEVNLDAEFR, 70 μM) and enzyme (1-5 μg protein). Reactions were incubated at 37 °C for various times and the reaction products were resolved by RP-HPLC using a linear gradient from 0-70 B over 30 minutes (A=0.1% TFA in water, B=).1%TFA/10%water/90%AcCN). The elution profile was monitored by absorbance at 214 nm. In preliminary experiments, the two product peaks which eluted before the intact peptide substrate, were confirmed to have the sequence DAEFR and SEVNL using both

What is claimed is:

1. Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for the peptide DTG, where the first nucleic acid of the first special set of nucleic acids is, the first special nucleic acid, and where the second set of nucleic acids code for either the peptide DSG or DTG, where the last nucleic acid of the second set of nucleic acids is the last special nucleic acid, with the proviso that the nucleic acids disclosed in SEQ ID NO. 1 and SEQ. ID NO. 5 are not included.

2. The nucleic acid polynucleotide of claim 1 where the two sets of nucleic acids are separated by nucleic acids that code for about 125 to 222 amino acid positions, which may be any amino acids.

3. The nucleic acid polynucleotide of claim 2 that code for about 150 to 172 amino acid positions, which may be any amino acids.

4. The nucleic acid polynucleotide of claim that code for about 172 amino acid positions, which may be any amino acids.

5. The nucleic acid polynucleotide of claim 4 where the nucleotides are described in SEQ. ID. NO. 3

6. The nucleic acid polynucleotide of claim 2 where the two sets of nucleic acids are separated by nucleic acids that code for about 150 to 196 amino acid positions.

7. The nucleic acid polynucleotide of claim 6 where the two sets of nucleotides are separated by nucleic acids that code for about 196 amino acids (positions).

16. Claims 1-15 where the last special nucleic acid is operably linked to any codon linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any reporter proteins or proteins which facilitate purification.

17. The nucleic acid polynucleotide of claims 1-16 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

18. * Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for DTG, where the first nucleic acid of the first special set of nucleic acids is, the first special nucleic acid, and where the second set of nucleic acids code for either DSG or DTG, where the last nucleic acid of the second set of special nucleic acids is the last special nucleic acid, where the first special nucleic acid is operably linked to nucleic acids that code for any number of amino acids from zero to 81 amino acids and where each of those codons may code for any amino acid.

19. The nucleic acid polynucleotide of claim 18 , where the first special nucleic acid is operably linked to nucleic acids that code for any number of from 64 to 77 amino acids where each codon may code for any amino acid.

20. The nucleic acid polynucleotide of claim 19 , where the first special nucleic acid is operably linked to nucleic acids that code for about 71 amino acids peptide.

21. The nucleic acid polynucleotide of claim 20, where the first special nucleic acid is operably linked to 71 amino acid peptide and where the first of those 71 amino acids is the amino acid T.

27. The nucleic acid polynucleotide of claim 22, where the polynucleotide comprises identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 35 or 47 amino acids, see Example 11 for the 47 example, beginning from the DTG site and including the nucleotides from that code for the previous 35 or 47 amino acids before the DTG site).

28. * Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for the peptide DTG, where the first nucleic acid of the first special set of amino acids is, the first special nucleic acid, and where the second set of special nucleic acids code for either the peptide DSG or DTG, where the last nucleic acid of the second set of special nucleic acids, the last special nucleic acid, is operably linked to nucleic acids that code for any number of codons from 50 to 170 codons.

29. The nucleic acid polynucleotide of claim 29 where the last special nucleic acid is operably linked to nucleic acids comprising from 100 to 170 codons.

30. The nucleic acid polynucleotide of claim 30 where the last special nucleic acid is operably linked to nucleic acids comprising from 142 to 163 codons.

31. The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 142 codons.

32. The nucleic acid polynucleotide of claim 32 where the polynucleotide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

43. A cell or cell line which contans a polynucleotide described in claims 1-42.

44. Any isolated or purified peptide or protein comprising an amino acid polymer that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid position can be any amino acid, where the first set of special amino acids consists of the peptide DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, where the second set of amino acids is selected from the peptide comprising either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, with the proviso that the proteases disclosed in SEQ ID NO. 2 and SEQ. ID NO. 6 are not included.

45. The amino acid polypeptide of claim 45 where the two sets of amino acids are separated by about 125 to 222 amino acid positions where in each position it may be any amino acid.

46. The amino acid polypeptide of claim 46 where the two sets of amino acids are separated by about 150 to 172 amino acids.

47. The amino acid polypeptide of claim 47 where the two sets of amino acids are separated by about 172 amino acids.

48. The amino acid polypeptide of claim 48 where the protease is described in SEQ. ID. NO. 4

49. The amino acid polypeptide of claim 46 where the two sets of amino acids are separated by about 150 to 196 amino acids.

50. The amino acid polypeptide of claim 50 where the two sets of amino acids are separated by about 196 amino acids.

59. Claims 45-59 where the last special amino acid is operably linked any peptide selected from the group consisting of any reporter proteins or proteins which facilitate purification.

60. The amino acid polypeptide of claims 45-60 where the first special amino acid is operably linked to any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

61. * Any isolated or purified peptide or protein comprising an amino acid polypeptide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino acid, where the first set of special amino acids consists of the amino acids DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids is either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, where the first special amino acid is operably linked to amino acids that code for any number of amino acids from zero to 81 amino acid positions where in each position it may be any amino acid.

62. The amino acid polypeptide of claim 62, where the first special amino acid is operably linked to a peptide from about 30 to 77 amino acids positions where each amino acid position may be any amino acid.

63. The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to a peptide of 35, 47, 71, or 77 amino acids.

64. The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to the same corresponding peptides from SEQ. ID. NO. 3 that are 35, 47, 71, or 77 peptides in length, beginning counting with the amino acids on the first special sequence, DTG, towards the N-terminal of SEQ. ID. NO. 3.

acid, where the first set of special amino acids consists of the amino acids that code for DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids are either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, which is operably linked to any number of amino acids from 50 to 170 amino acids, which may be any amino acids.

73. The amino acid polypeptide of claim 73 where the last special amino acid is operably linked to a peptide of about 100 to 170 amino acids.

74. The amino acid polypeptide of claim 74 where the last special amino acid is operably linked to to a peptide of about 142 to 163 amino acids.

75. The amino acid polypeptide of claim 75 where the last special amino acid is operably linked to to a peptide of about about 142 amino acids.

76. The amino acid polypeptide of claim 76 where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

77. The amino acid polypeptide of claim 75 where the last special amino acid is operably linked to a peptide of about 163 amino acids.

78. The amino acid polypeptide of claim 79 where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

79. The amino acid polypeptide of claim 79, where the complete polypeptide comprises SEQ. ID. # (Example 9 or 10).

80. The amino acid polypeptide of claim 74 where the last special amino acid is operably linked to to a peptide of about 170 amino acids.

81. Claim 46-81 where the second set of special amino acids is comprised of the peptide with the amino acid sequence DSG.

(a) a nucleotide sequence encoding a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), wherein said Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) polypeptides have the complete amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID No:6, respectively; and

(b) a nucleotide sequence complementary to the nucleotide sequence of (a).

92. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp1, and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO:1.

93. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp2(a), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO:4.

94. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp2(b), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO:5.

95. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a) or (b) of claim 92.

96. A vector comprising the nucleic acid molecule of claim 96.

97. The vector of claim 97, wherein said nucleic acid molecule is operably linked to a promoter for the expression of a Hu-Asp polypeptide.

98. The vector of claim 97, wherein said Hu-Asp polypeptide is Hu-Asp1.

99. The vector of claim 97, wherein said Hu-Asp polypeptide is Hu-Asp2(a).

100. The vector of claim 97, wherein said Hu-Asp polypeptide is Hu-Asp2(b).

109. The method of claim 108 where the cells are collected and the critical peptide is the APP C-terminal peptide created as a result of the β secretase cleavage.

110. The method of claim 108 where the supernantent is collected and the critical peptide is soluble APP where the soluble APP has a C-terminal created by β secretase cleavage.

111. The method of claim 108 where the cells contain any of the nucleic acids or polypeptides of claims 1-86 and where the cells are shown to cleave the β secretase site of any peptide having the following peptide structure, P2, P1, P1', P2', where P2 is K or N, where P1 is M or L, where P1' is D, where P2' is A.

112. The method of claim 111 where P2 is K and P1 is M.

113 The method of claim 112 where P2 is N and P1 is L.

114 * Any bacterial cell comprising any nucleic acids or peptides in claims 1-86 and 92-107.

115 A bacterial cell of claim 114 where the bacteria is *E coli*.

116 Any eukaryotic cell comprising any nucleic acids or polypeptides in claims 1-86 and 92-107.

117 * Any insect cell comprising any of the nucleic acids or polypeptides in claims 1-86 and 92-107.

118 A insect cell of claim 117 where the insect is sf9, or High 5.

119 A insect cell of claim 100 where the insect cell is High 5.

120 A mammalian cell comprising any of the nucleic acids or polypeptides in claims 1-86 and 92-107.

134 Any eukaryotic cell line, comprising nucleic acids or polypeptides of claim 130-133.

135 Any cell line of claim 134 that is a mammaliam cell line (HEK293, Neuro2a, are preferred plus any others.)

136 A method for identifying inhibitors of an enzyme that cleaves the beta secretase cleavabe site of APP comprising:

a) culturing cells in a culture medium under conditions in which the enzyme causes processing of APP and release of amyloid beta-peptide into the medium and causes the accumulation of CTF99 fragments of APP in cell lysates,

b) exposing the cultured cells to a test compound; and specifically determining whether the test compound inhibits the function of the enzyme by measuring the amount of amyloid beta-peptide released into the medium and or the amount of CTF99 fragments of APP in cell lysates;

c) identifying test compounds diminishing the amount of soluble amyloid beta peptide present in the culture medium and diminution of CTF99 fragments of APP in cell lysates as Asp2 inhibitors.

137 The method of claim 136 wherein the cultured cells are a human, rodent or insect cell line.

138 The method of claim 137 wherein the human or rodent cell line exhibits β secretase activity in which processing of APP occurs with release of amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates.

139. A method as in claim 138 wherein the human or rodent cell line treated with the antisense oligomers directed against the enzyme that exhibits β secretase activity, reduces release of soluble amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates.

FIGURE 1 (1)

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ATGGGCGCACTGGCCCGGGCGCTGCTGCTGCCTCTGCTGGCCCAGTGGCTCCTGCGCGCC
M  G  A  L  A  R  A  L  L  L  P  L  L  A  Q  W  L  L  R  A

CCCCGGAGCTGGCCCCCGCGCCCTTCACGCTGCCCCTCCGGGTGGCCGCGGCCACGAAC
A  P  E  L  A  P  A  P  F  T  L  P  L  R  V  A  A  A  T  N

CGCGTAGTTGCGCCCACCCCGGGACCCGGGACCCCTGCCGAGCGCCACGCCGACGGCTTG
R  V  V  A  P  T  P  G  P  G  T  P  A  E  R  H  A  D  G  L

GCGCTCGCCCTGGAGCCTGCCCTGGCGTCCCCCGCGGGCGCCGCCAACTTCTTGGCCATG
A  L  A  L  E  P  A  L  A  S  P  A  G  A  A  N  F  L  A  M

GTAGACAACCTGCAGGGGGACTCTGGCCGCGGCTACTACCTGGAGATGCTGATCGGGACC
V  D  N  L  Q  G  D  S  G  R  G  Y  Y  L  E  M  L  I  G  T

CCCCCGCAGAAGCTACAGATTCTCGTTGACACTGGAAGCAGTAACTTTGCCGTGGCAGGA
P  P  Q  K  L  Q  I  L  V  D  T  G  S  S  N  F  A  V  A  G

ACCCCGCACTCCTACATAGACACGTACTTTGACACAGAGAGGTCTAGCACATACCGCTCC
T  P  H  S  Y  I  D  T  Y  F  D  T  E  R  S  S  T  Y  R  S

AAGGGCTTTGACGTCACAGTGAAGTACACACAAGGAAGCTGGACGGGCTTCGTTGGGGAA
K  G  F  D  V  T  V  K  Y  T  Q  G  S  W  T  G  F  V  G  E

GACCTCGTCACCATCCCCAAAGGCTTCAATACTTCTTTTCTTGTCAACATTGCCACTATT
D  L  V  T  I  P  K  G  F  N  T  S  F  L  V  N  I  A  T  I

TTTGAATCAGAGAATTTCTTTTTGCCTGGGATTAAATGGAATGGAATACTTGGCCTAGCT
F  E  S  E  N  F  F  L  P  G  I  K  W  N  G  I  L  G  L  A

TATGCCACACTTGCCAAGCCATCAAGTTCTCTGGAGACCTTCTTCGACTCCCTGGTGACA
Y  A  T  L  A  K  P  S  S  S  L  E  T  F  F  D  S  L  V  T

CAAGCAAACATCCCCAACGTTTTCTCCATGCAGATGTGTGGAGCCGGCTTGCCCGTTGCT
Q  A  N  I  P  N  V  F  S  M  Q  M  C  G  A  G  L  P  V  A

GGATCTGGGACCAACGGAGGTAGTCTTGTCTTGGGTGGAATTGAACCAAGTTTGTATAAA
G  S  G  T  N  G  G  S  L  V  L  G  G  I  E  P  S  L  Y  K

GGAGACATCTGGTATACCCCTATTAAGGAAGAGTGGTACTACCAGATAGAAATTCTGAAA
G  D  I  W  Y  T  P  I  K  E  E  W  Y  Y  Q  I  E  I  L  K

TTGGAAATTGGAGGCCAAAGCCTTAATCTGGACTGCAGAGAGTATAACGCAGACAAGGCC
L  E  I  G  G  Q  S  L  N  L  D  C  R  E  Y  N  A  D  K  A

ATCGTGGACAGTGGCACCACGCTGCTGCGCCTGCCCCAGAAGGTGTTTGATGCGGTGGTG
I  V  D  S  G  T  T  L  L  R  L  P  Q  K  V  F  D  A  V  V

GAAGCTGTGGCCCGCGCATCTCTGATTCCAGAATTCTCTGATGGTTTCTGGACTGGGTCC
E  A  V  A  R  A  S  L  I  P  E  F  S  D  G  F  W  T  G  S

CAGCTGGCGTGCTGGACGAATTCGGAAACACCTTGGTCTTACTTCCCTAAAATCTCCATC
Q  L  A  C  W  T  N  S  E  T  P  W  S  Y  F  P  K  I  S  I

TACCTGAGAGATGAGAACTCCAGCAGGTCATTCCGTATCACAATCCTGCCTCAGCTTTAC
Y  L  R  D  E  N  S  S  R  S  F  R  I  T  I  L  P  Q  L  Y

ATTCAGCCCATGATGGGGGCCGGCCTGAATTATGAATGTTACCGATTCGGCATTTCCCCA
I  Q  P  M  M  G  A  G  L  N  Y  E  C  Y  R  F  G  I  S  P

TCCACAAATGCGCTGGTGATCGGTGCCACGGTGATGGAGGGCTTCTACGTCATCTTCGAC
S  T  N  A  L  V  I  G  A  T  V  M  E  G  F  Y  V  I  F  D

AGAGCCCAGAAGAGGGTGGGCTTCGCAGCGAGCCCCTGTGCAGAAATTGCAGGTGCTGCA
```

FIGURE 2 (1)

```
ATGGCCCAAGCCCTGCCCTGGCTCCTGCTGTGGATGGGCGCGGGAGTGCTGCCTGCCCAC
M  A  Q  A  L  P  W  L  L  L  W  M  G  A  G  V  L  P  A  H

GGCACCCAGCACGGCATCCGGCTGCCCCTGCGCAGCGGCCTGGGGGGCGCCCCCCTGGGG
G  T  Q  H  G  I  R  L  P  L  R  S  G  L  G  G  A  P  L  G

CTGCGGCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTT
L  R  L  P  R  E  T  D  E  E  P  E  E  P  G  R  R  G  S  F

GTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACC
V  E  M  V  D  N  L  R  G  K  S  G  Q  G  Y  Y  V  E  M  T

GTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCA
V  G  S  P  P  Q  T  L  N  I  L  V  D  T  G  S  S  N  F  A

GTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q  R  Q  L  S  S  T

TACCGGGACCTCCGGAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAG
Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  G  K  W  E  G  E

CTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L  G  T  D  L  V  S  I  P  H  G  P  N  V  T  V  R  A  N  I

GCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTG
A  A  I  T  E  S  D  K  F  F  I  N  G  S  N  W  E  G  I  L

GGGCTGGCCTATGCTGAGATTGCCAGGCTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCT
G  L  A  Y  A  E  I  A  R  L  C  G  A  G  F  P  L  N  Q  S

GAAGTGCTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATCGACCACTCGCTGTAC
E  V  L  A  S  V  G  G  S  M  I  I  G  G  I  D  H  S  L  Y

ACAGGCAGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTATGAGGTGATCATTGTG
T  G  S  L  W  Y  T  P  I  R  R  E  W  Y  Y  E  V  I  I  V

CGGGTGGAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAGTACAACTATGACAAG
R  V  E  I  N  G  Q  D  L  K  M  D  C  K  E  Y  N  Y  D  K

AGCATTGTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCA
S  I  V  D  S  G  T  T  N  L  R  L  P  K  K  V  F  E  A  A

GTCAAATCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGATGGTTTCTGGCTAGGA
V  K  S  I  K  A  A  S  S  T  E  K  F  P  D  G  F  W  L  G

GAGCAGCTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATTTTCCCAGTCATCTCA
E  Q  L  V  C  W  Q  A  G  T  T  P  W  N  I  F  P  V  I  S

CTCTACCTAATGGGTGAGGTTACCAACCAGTCCTTCCGCATCACCATCCTTCCGCAGCAA
L  Y  L  M  G  E  V  T  N  Q  S  F  R  I  T  I  L  P  Q  Q

TACCTGCGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGTTACAAGTTTGCCATC
```

FIGURE 3 (1)

```
ATGGCCCAAGCCCTGCCCTGGCTCCTGCTGTGGATGGGCGCGGGAGTGCTGCCTGCCCAC
M  A  Q  A  L  P  W  L  L  L  W  M  G  A  G  V  L  P  A  H

GGCACCCAGCACGGCATCCGGCTGCCCCTGCGCAGCGGCCTGGGGGGCGCCCCCCTGGGG
G  T  Q  H  G  I  R  L  P  L  R  S  G  L  G  G  A  P  L  G

CTGCGGCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTT
L  R  L  P  R  E  T  D  E  E  P  E  E  P  G  R  R  G  S  F

GTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACC
V  E  M  V  D  N  L  R  G  K  S  G  Q  G  Y  Y  V  E  M  T

GTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCA
V  G  S  P  P  Q  T  L  N  I  L  V  D  T  G  S  S  N  F  A

GTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q  R  Q  L  S  S  T

TACCGGGACCTCCGGAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAG
Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  G  K  W  E  G  E

CTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L  G  T  D  L  V  S  I  P  H  G  P  N  V  T  V  R  A  N  I

GCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTG
A  A  I  T  E  S  D  K  F  F  I  N  G  S  N  W  E  G  I  L

GGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTGGAGCCTTTCTTTGACTCT
G  L  A  Y  A  E  I  A  R  P  D  D  S  L  E  P  F  F  D  S

CTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTC
L  V  K  Q  T  H  V  P  N  L  F  S  L  Q  L  C  G  A  G  F

CCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATC
P  L  N  Q  S  E  V  L  A  S  V  G  G  S  M  I  I  G  G  I

GACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTAT
D  H  S  L  Y  T  G  S  L  W  Y  T  P  I  R  R  E  W  Y  Y

GAGGTCATCATTGTGCGGGTGGAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAG
E  V  I  I  V  R  V  E  I  N  G  Q  D  L  K  M  D  C  K  E

TACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAA
Y  N  Y  D  K  S  I  V  D  S  G  T  T  N  L  R  L  P  K  K

GTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGAT
V  F  E  A  A  V  K  S  I  K  A  A  S  S  T  E  K  F  P  D
```

FIGURE 4

```
ATGGCCCCAGCGCTGCACTGGCTCCTGCTATGGGTGGGCTCGGGAATGCTGCCTGCCCAG
M  A  P  A  L  H  W  L  L  L  W  V  G  S  G  M  L  P  A  Q
GGAACCCATCTCGGCATCCGGCTGCCCCTTCGCAGCGGCCTGGCAGGGCCACCCCTGGGC
G  T  H  L  G  I  R  L  P  L  R  S  G  L  A  G  P  P  L  G
CTGAGGCTGCCCCCGGGAGACTGACGAGGAATCGGAGGAGCCTGGCCGGAGAGGCAGCTTT
L  R  L  P  R  E  T  D  E  E  S  E  E  P  G  R  R  G  S  F
GTGGAGATGGTGGACAACCTGAGGGGAAAGTCCGGCCAGGGCTACTATGTGGAGATGACC
V  E  M  V  D  N  L  R  G  K  S  G  Q  G  Y  Y  V  E  M  T
GTAGGCAGCCCCCCACAGACGCTCAACATCCTGGTGGACACGGGCAGTAGTAACTTTGCA
V  G  S  P  P  Q  T  L  N  I  L  V  D  T  G  S  S  N  F  A
GTGGGGGCTGCCCCACACCCTTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q  R  Q  L  S  S  T
TATCGAGACCTCCGAAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAGGGGGAA
Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  G  K  W  E  G  E
CTGGGCACCGACCTGGTGAGCATCCCTCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L  G  T  D  L  V  S  I  P  H  G  P  N  V  T  V  R  A  N  I
GCTGCCATCACTGAATCGGACAAGTTCTTCATCAATGGTTCCAACTGGGAGGGCATCCTA
A  A  I  T  E  S  D  K  F  F  I  N  G  S  N  W  E  G  I  L
GGGCTGGCCTATGCTGAGATTGCCAGGCCCGACGACTCTTTGGAGCCCTTCTTTGACTCC
G  L  A  Y  A  E  I  A  R  P  D  D  S  L  E  P  F  F  D  S
CTGGTGAAGCAGACCCACATTCCCAACATCTTTTCCCTGCAGCTCTGTGGCGCTGGCTTC
L  V  K  Q  T  H  I  P  N  I  F  S  L  Q  L  C  G  A  G  F
CCCCTCAACCAGACCGAGGCACTGGCCTCGGTGGGAGGGAGCATGATCATTGGTGGTATC
P  L  N  Q  T  E  A  L  A  S  V  G  G  S  M  I  I  G  G  I
GACCACTCGCTATACACGGGCAGTCTCTGGTACACACCCATCCGGCGGGAGTGGTATTAT
D  H  S  L  Y  T  G  S  L  W  Y  T  P  I  R  R  E  W  Y  Y
GAAGTGATCATTGTACGTGTGGAAATCAATGGTCAAGATCTCAAGATGGACTGCAAGGAG
E  V  I  I  V  R  V  E  I  N  G  Q  D  L  K  M  D  C  K  E
TACAACTACGACAAGAGCATTGTGGACAGTGGGACCACCAACCTTCGCTTGCCCAAGAAA
Y  N  Y  D  K  S  I  V  D  S  G  T  T  N  L  R  L  P  K  K
GTATTTGAAGCTGCCGTCAAGTCCATCAAGGCAGCCTCCTCGACGGAGAAGTTCCCGGAT
V  F  E  A  A  V  K  S  I  K  A  A  S  S  T  E  K  F  P  D
GGCTTTTGGCTAGGGGAGCAGCTGGTGTGCTGGCAAGCAGGCACGACCCCTTGGAACATT
G  F  W  L  G  E  Q  L  V  C  W  Q  A  G  T  T  P  W  N  I
TTCCCAGTCATTTCACTTTACCTCATGGGTGAAGTCACCAATCAGTCCTTCCGCATCACC
F  P  V  I  S  L  Y  L  M  G  E  V  T  N  Q  S  F  R  I  T
ATCCTTCCTCAGCAATACCTACGGCCGGTGGAGGACGTGGCCACGTCCCAAGACGACTGT
I  L  P  Q  Q  Y  L  R  P  V  E  D  V  A  T  S  Q  D  D  C
TACAAGTTCGCTGTCTCACAGTCATCCACGGGCACTGTTATGGGAGCCGTCATCATGGAA
Y  K  F  A  V  S  Q  S  S  T  G  T  V  M  G  A  V  I  M  E
GGTTTCTATGTCGTCTTCGATCGAGCCCGAAAGCGAATTGGCTTTGCTGTCAGCGCTTGC
G  F  Y  V  V  F  D  R  A  R  K  R  I  G  F  A  V  S  A  C
CATGTGCACGATGAGTTCAGGACGGCGGCAGTGGAAGGTCCGTTTGTTACGGCAGACATG
H  V  H  D  E  F  R  T  A  A  V  E  G  P  F  V  T  A  D  M
GAAGACTGTGGCTACAACATTCCCCAGACAGATGAGTCAACACTTATGACCATAGCCTAT
E  D  C  G  Y  N  I  P  Q  T  D  E  S  T  L  M  T  I  A  Y
GTCATGGCGGCCATCTGCGCCCTCTTCATGTTGCCACTCTGCCTCATGGTATGTCAGTGG
V  M  A  A  I  C  A  L  F  M  L  P  L  C  L  M  V  C  Q  W
CGCTGCCTGCGTTGCCTGCGCCACCAGCACGATGACTTTGCTGATGACATCTCCCTGCTC
R  C  L  R  C  L  R  H  Q  H  D  D  F  A  D  D  I  S  L  L
AAGTAAGGAGGCTCGTGGGCAGATGATGGAGACGCCCCTGGACCACATCTGGGTGGTTCC
K
CTTTGGTCACATGAGTTGGAGCTATGGATGGTACCTGTGGCCAGAGCACCTCAGGACCCT
CACCAACCTGCCAATGCTTCTGGCGTGACAGAACAGAGAAATCAGGCAAGCTGGATTACA
GGGCTTGCACCTGTAGGACACAGGAGAGGGAAGGAAGCAGCGTTCTGGTGGCAGGAATAT
CCTTAGGCACCACAAACTTGAGTTGGAAATTTTGCTGCTTGAAGCTTCAGCCCTGACCCT
CTGCCCAGCATCCTTTAGAGTCTCCAACCTAAAGTATTCTTTATGTCCTTCCAGAAGTAC
TGGCGTCATACTCAGGCTACCCGGCATGTGTCCCTGTGGTACCCTGGCAGAGAAAGGGCC
AATCTCATTCCCTGCTGGCCAAAGTCAGCAGAAGAAGGTGAAGTTTGCCAGTTGCTTTAG
TGATAGGGACTGCAGACTCAAGCCTACACTGGTACAAAGACTGCGTCTTGAGATAAACAA
GAA
```

FIGURE 6 (1)

```
ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCACCCAGCACGGCATCCGG
M  A  S  M  T  G  G  Q  Q  M  G  R  G  S  T  Q  H  G  I  R

CTGCCCCTGCGCAGCGGCCTGGGGGGCGCCCCCCTGGGGCTGCGGCTGCCCCGGGAGACC
L  P  L  R  S  G  L  G  G  A  P  L  G  L  R  L  P  R  E  T

GACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTTGTGGAGATGGTGGACAACCTG
D  E  E  P  E  E  P  G  R  R  G  S  F  V  E  M  V  D  N  L

AGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACCGTGGGCAGCCCCCCGCAGACG
R  G  K  S  G  Q  G  Y  Y  V  E  M  T  V  G  S  P  P  Q  T

CTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCAGTGGGTGCTGCCCCCCACCCC
L  N  I  L  V  D  T  G  S  S  N  F  A  V  G  A  A  P  H  P

TTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACATACCGGGACCTCCGGAAGGGC
F  L  H  R  Y  Y  Q  R  Q  L  S  S  T  Y  R  D  L  R  K  G

GTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAGCTGGGCACCGACCTGGTAAGC
V  Y  V  P  Y  T  Q  G  K  W  E  G  E  L  G  T  D  L  V  S

ATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATTGCTGCCATCACTGAATCAGAC
I  P  H  G  P  N  V  T  V  R  A  N  I  A  A  I  T  E  S  D

AAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTGGGGCTGGCCTATGCTGAGATT
K  F  F  I  N  G  S  N  W  E  G  I  L  G  L  A  Y  A  E  I

GCCAGGCCTGACGACTCCCTGGAGCCTTTCTTTGACTCTCTGGTAAAGCAGACCCACGTT
A  R  P  D  D  S  L  E  P  F  F  D  S  L  V  K  Q  T  H  V

CCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCTGAAGTG
P  N  L  F  S  L  Q  L  C  G  A  G  F  P  L  N  Q  S  E  V

CTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATCGACCACTCGCTGTACACAGGC
L  A  S  V  G  G  S  M  I  I  G  G  I  D  H  S  L  Y  T  G

AGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTATGAGGTCATCATTGTGCGGGTG
S  L  W  Y  T  P  I  R  R  E  W  Y  Y  E  V  I  I  V  R  V

GAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATT
E  I  N  G  Q  D  L  K  M  D  C  K  E  Y  N  Y  D  K  S  I

GTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCAGTCAAA
V  D  S  G  T  T  N  L  R  L  P  K  K  V  F  E  A  A  V  K

TCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGATGGTTTCTGGCTAGGAGAGCAG
S  I  K  A  A  S  S  T  E  K  F  P  D  G  F  W  L  G  E  Q

CTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATTTTCCCAGTCATCTCACTCTAC
L  V  C  W  Q  A  G  T  T  P  W  N  I  F  P  V  I  S  L  Y

CTAATGGGTGAGGTTACCAACCAGTCCTTCCGCATCACCATCCTTCCGCAGCAATACCTG
L  M  G  E  V  T  N  Q  S  F  R  I  T  I  L  P  Q  Q  Y  L

CGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGTTACAAGTTTGCCATCTCACAG
```

FIGURE 7 (1)

```
ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCGATGACTATCTCTGACTCT
M  A  S  M  T  G  G  Q  Q  M  G  R  G  S  M  T  I  S  D  S

CCGCGTGAACAGGACGGATCCACCCAGCACGGCATCCGGCTGCCCCTGCGCAGCGGCCTG
P  R  E  Q  D  G  S  T  Q  H  G  I  R  L  P  L  R  S  G  L

GGGGGCGCCCCCCTGGGGCTGCGGCTGCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCC
G  G  A  P  L  G  L  R  L  P  R  E  T  D  E  E  P  E  E  P

GGCCGGAGGGGCAGCTTTGTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGC
G  R  R  G  S  F  V  E  M  V  D  N  L  R  G  K  S  G  Q  G

TACTACGTGGAGATGACCGTGGGCAGCCCCCCCGCAGACGCTCAACATCCTGGTGGATACA
Y  Y  V  E  M  T  V  G  S  P  P  Q  T  L  N  I  L  V  D  T

GGCAGCAGTAACTTTGCAGTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAG
G  S  S  N  F  A  V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q

AGGCAGCTGTCCAGCACATACCGGGACCTCCGGAAGGGCGTGTATGTGCCCTACACCCAG
R  Q  L  S  S  T  Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q

GGCAAGTGGGAAGGGGAGCTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTC
G  K  W  E  G  E  L  G  T  D  L  V  S  I  P  H  G  P  N  V

ACTGTGCGTGCCAACATTGCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCC
T  V  R  A  N  I  A  A  I  T  E  S  D  K  F  F  I  N  G  S

AACTGGGAAGGCATCCTGGGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTG
N  W  E  G  I  L  G  L  A  Y  A  E  I  A  R  P  D  D  S  L

GAGCCTTTCTTTGACTCTCTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAG
E  P  F  F  D  S  L  V  K  Q  T  H  V  P  N  L  F  S  L  Q

CTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAGC
L  C  G  A  G  F  P  L  N  Q  S  E  V  L  A  S  V  G  G  S

ATGATCATTGGAGGTATCGACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATC
M  I  I  G  G  I  D  H  S  L  Y  T  G  S  L  W  Y  T  P  I

CGGCGGGAGTGGTATTATGAGGTCATCATTGTGCGGGTGGAGATCAATGGACAGGATCTG
R  R  E  W  Y  Y  E  V  I  I  V  R  V  E  I  N  G  Q  D  L

AAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAAC
K  M  D  C  K  E  Y  N  Y  D  K  S  I  V  D  S  G  T  T  N

CTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTCC
L  R  L  P  K  K  V  F  E  A  A  V  K  S  I  K  A  A  S  S

ACGGAGAAGTTCCCTGATGGTTTCTGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGC
T  E  K  F  P  D  G  F  W  L  G  E  Q  L  V  C  W  Q  A  G

ACCACCCCTTGGAACATTTTCCCAGTCATCTCACTCTACCTAATGGGTGAGGTTACCAAC
T  T  P  W  N  I  F  P  V  I  S  L  Y  L  M  G  E  V  T  N
```

FIGURE 8 (1)

```
ATGACTCAGCATGGTATTCGTCTGCCACTGCGTAGCGGTCTGGGTGGTGCTCCACTGGGT
M  T  Q  H  G  I  R  L  P  L  R  S  G  L  G  G  A  P  L  G  -

CTGCGTCTGCCCCCGGGAGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTT
L  R  L  P  R  E  T  D  E  E  P  E  E  P  G  R  R  G  S  F  -

GTGGAGATGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACC
V  E  M  V  D  N  L  R  G  K  S  G  Q  G  Y  Y  V  E  M  T  -

GTGGGCAGCCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCA
V  G  S  P  P  Q  T  L  N  I  L  V  D  T  G  S  S  N  F  A  -

GTGGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCACA
V  G  A  A  P  H  P  F  L  H  R  Y  Y  Q  R  Q  L  S  S  T  -

TACCGGGACCTCCGGAAGGGCGTGTATGTGCCCTACACCCAGGGCAAGTGGGAAGGGGAG
Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  G  K  W  E  G  E  -

CTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCGTGCCAACATT
L  G  T  D  L  V  S  I  P  H  G  P  N  V  T  V  R  A  N  I  -

GCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGGGAAGGCATCCTG
A  A  I  T  E  S  D  K  F  F  I  N  G  S  N  W  E  G  I  L  -

GGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTGGAGCCTTTCTTTGACTCT
G  L  A  Y  A  E  I  A  R  P  D  D  S  L  E  P  F  F  D  S

CTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTC
L  V  K  Q  T  H  V  P  N  L  F  S  L  Q  L  C  G  A  G  F  -

CCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAGCATGATCATTGGAGGTATC
P  L  N  Q  S  E  V  L  A  S  V  G  G  S  M  I  I  G  G  I  -

GACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATCCGGCGGGAGTGGTATTAT
D  H  S  L  Y  T  G  S  L  W  Y  T  P  I  R  R  E  W  Y  Y  -

GAGGTCATCATTGTGCGGGTGGAGATCAATGGACAGGATCTGAAAATGGACTGCAAGGAG
E  V  I  I  V  R  V  E  I  N  G  Q  D  L  K  M  D  C  K  E

TACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAACCTTCGTTTGCCCAAGAAA
Y  N  Y  D  K  S  I  V  D  S  G  T  T  N  L  R  L  P  K  K  -

GTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTCCACGGAGAAGTTCCCTGAT
V  F  E  A  A  V  K  S  I  K  A  A  S  S  T  E  K  F  P  D  -

GGTTTCTGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATT
G  F  W  L  G  E  Q  L  V  C  W  Q  A  G  T  T  P  W  N  I  -

TTCCCAGTCATCTCACTCTACCTAATGGGTGAGGTTACCAACCAGTCCTTTCGCATCACC
F  P  V  I  S  L  Y  L  M  G  E  V  T  N  Q  S  F  R  I  T  -

ATCCTTCCGCAGCAATACCTGCGGCCAGTGGAAGATGTGGCCACGTCCCAAGACGACTGT
I  L  P  Q  Q  Y  L  R  P  V  E  D  V  A  T  S  Q  D  D  C  -
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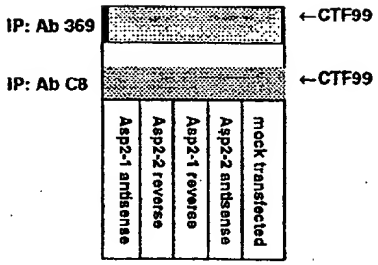
FIGURE 9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←CTF99
IP: Ab C8
←CTF99
Asp2-1 antisense
Asp2-2 reverse
Asp2-1 reverse
Asp2-2 antisense
mock transfected

FIGURE 11

MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLPRETDEE
PEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFA
VGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSIPH
GPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDS
LVKQTHVPNLFSLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGSLW
YTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRLPKK
VFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMG
EVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIME
GFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQT
DES

SEQUENCE LISTING

<110> Gurney, Mark E.
Bienkowski, Michael J.
Heinrikson, Robert L.
Parodi, Luis A.
Yan, Riqiang
Pharmacia & Upjohn Company

<120> Alzheimer's Disease Secretase

<130> 6177.P CP

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<151> 1998-09-24

<160> 49

<170> PatentIn Ver. 2.0

<210> 1

<211> 1804

<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

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20 25 30

Leu Arg Val Ala Ala Ala Thr Asn Arg Val Val Ala Pro Thr Pro Gly
35 40 45

Pro Gly Thr Pro Ala Glu Arg His Ala Asp Gly Leu Ala Leu Ala Leu
50 55 60

Glu Pro Ala Leu Ala Ser Pro Ala Gly Ala Ala Asn Phe Leu Ala Met
65 70 75 80

Val Asp Asn Leu Gln Gly Asp Ser Gly Arg Gly Tyr Tyr Leu Glu Met
85 90 95

Leu Ile Gly Thr Pro Pro Gln Lys Leu Gln Ile Leu Val Asp Thr Gly
100 105 110

Ser Ser Asn Phe Ala Val Ala Gly Thr Pro His Ser Tyr Ile Asp Thr
115 120 125

Tyr Phe Asp Thr Glu Arg Ser Ser Thr Tyr Arg Ser Lys Gly Phe Asp
130 135 140

Gly Thr Thr Leu Leu Arg Leu Pro Gln Lys Val Phe Asp Ala Val Val
305 310 315 320

Glu Ala Val Ala Arg Ala Ser Leu Ile Pro Glu Phe Ser Asp Gly Phe
325 330 335

Trp Thr Gly Ser Gln Leu Ala Cys Trp Thr Asn Ser Glu Thr Pro Trp
340 345 350

Ser Tyr Phe Pro Lys Ile Ser Ile Tyr Leu Arg Asp Glu Asn Ser Ser
355 360 365

Arg Ser Phe Arg Ile Thr Ile Leu Pro Gln Leu Tyr Ile Gln Pro Met
370 375 380

Met Gly Ala Gly Leu Asn Tyr Glu Cys Tyr Arg Phe Gly Ile Ser Pro
385 390 395 400

Ser Thr Asn Ala Leu Val Ile Gly Ala Thr Val Met Glu Gly Phe Tyr
405 410 415

Val Ile Phe Asp Arg Ala Gln Lys Arg Val Gly Phe Ala Ala Ser Pro
420 425 430

Cys Ala Glu Ile Ala Gly Ala Ala Val Ser Glu Ile Ser Gly Pro Phe
435 440 445

Ser Thr Glu Asp Val Ala Ser Asn Cys Val Pro Ala Gln Ser Leu Ser
450 455 460

Glu Pro Ile Leu Trp Ile Val Ser Tyr Ala Leu Met Ser Val Cys Gly

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tacaactatg acaagagcat tgtggacagt ggcaccacca accttcgttt gcccaagaaa 900

gtgtttgaag ctgcagtcaa atccatcaag gcagcctcct ccacggagaa gttccctgat 960

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ccacccacca aatgcctctg ccttgatgga gaaggaaaag gctggcaagg tgggttccag 1680

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cttggtcacc tcaaatttaa gtcgggaaat tctgctgctt gaaacttcag ccctgaacct 1800

ttgtccacca ttcctttaaa ttctccaacc caaagtattc ttcttttctt agtttcagaa 1860

gtactggcat cacacgcagg ttaccttggc gtgtgtccct gtggtaccct ggcagagaag 1920

agaccaagct tgtttccctg ctggccaaag tcagtaggag aggatgcaca gtttgctatt 1980

tgctttagag acagggactg tataaacaag cctaacattg gtgcaaagat tgcctcttga 2040

attaaaaaaa aaaaaaaaaa aaaaaaaaaa 2070

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<212> PRT

<213> Homo sapiens

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Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro
195 200 205

Asn Leu Phe Ser Leu His Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
210 215 220

Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225 230 235 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
245 250 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
260 265 270

Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
275 280 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
290 295 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305 310 315 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
325 330 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val

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<211> 1977

<212> DNA

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ctgcggctgc cccgggagac cgacgaagag cccgaggagc ccggccggag gggcagcttt 180

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gtgggtgctg ccccccaccc cttcctgcat cgctactacc agaggcagct gtccagcaca 360

taccgggacc tccggaaggg tgtgtatgtg ccctacaccc agggcaagtg ggaaggggag 420

ctgggcaccg acctggtaag catcccccat ggccccaacg tcactgtgcg tgccaacatt 480

gctgccatca ctgaatcaga caagttcttc atcaacggct ccaactggga aggcatcctg 540

gggctggcct atgctgagat tgccaggctt tgtggtgctg gcttcccccc caaccagtct 600

gaagtgctgg cctctgtcgg agggagcatg atcattggag gtatcgacca ctcgctgtac 660

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tacctgcggc cagtggaaga tgtggccacg tcccaagacg actgttacaa gtttgccatc 1080

tcacagtcat ccacgggcac tgttatggga gctgttatca tggagggctt ctacgttgtc 1140

tttgatcggg cccgaaaacg aattggcttt gctgtcagcg cttgccatgt gcacgatgag 1200

ttcaggacgg cagcggtgga aggccctttt gtcaccttgg acatggaaga ctgtggctac 1260

aacattccac agacagatga gtcaaccctc atgaccatag cctatgtcat ggctgccatc 1320

tgcgccctct tcatgctgcc actctgcctc atggtgtgtc agtggcgctg cctccgctgc 1380

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Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160

Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp
                165                 170                 175

Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Leu Cys Gly
            180                 185                 190

Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly
        195                 200                 205

Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu
    210                 215                 220

Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val
225                 230                 235                 240
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405 410 415

Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser Thr Leu Met Thr
420 425 430

Ile Ala Tyr Val Met Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu
435 440 445

Cys Leu Met Val Cys Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Gln
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His Asp Asp Phe Ala Asp Asp Ile Ser Leu Leu Lys
465 470 475

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<211> 2043

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<213> Mus musculus

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            20                  25                  30

Gly Leu Ala Gly Pro Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
        35                  40                  45

Glu Glu Ser Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
    50                  55                  60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65                  70                  75                  80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
                85                  90                  95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
            100                 105                 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
        115                 120                 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
    130                 135                 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
145                 150                 155                 160
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325 330 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
340 345 350

Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg
355 360 365

Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala
370 375 380

Val Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu
385 390 395 400

Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala
405 410 415

Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu
420 425 430

Gly Pro Phe Val Thr Ala Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
435 440 445

Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala
450 455 460

Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys Gln Trp
465 470 475 480

Arg Cys Leu Arg Cys Leu Arg His Gln His Asp Asp Phe Ala Asp Asp
485 490 495

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cgcatggtgg atcccaagaa agccgctcag atccggtccc aggttatgac acacctccgt 1380

gtgatttatg agcgcatgaa tcagtctctc tccctgctct acaacgtgcc tgcagtggcc 1440

gaggagattc aggatgaagt tgatgagctg cttcagaaag agcaaaacta ttcagatgac 1500

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<211> 695

<212> PRT

<213> Homo sapiens

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Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

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Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
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Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685

Phe Phe Glu Gln Met Gln Asn
    690                 695
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tctttgaccg aaacgaaaac caccgtggag ctccttcccg tgaatggaga gttcagcctg 1620

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gtggaggttg acgccgctgt caccccagag gagcgccacc tgtccaagat gcagcagaac 2040

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<212> PRT

<213> Homo sapiens

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20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590

Glu Val Asn Leu Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn
690 695

<210> 13

<211> 2088

<212> DNA

<213> Homo sapiens

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atcttcatca ccttggtgat gctgaagaag aaacagtaca catccattca tcatggtgtg 1980

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<211> 695

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20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val

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Ala Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430
```

595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Phe Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn
690 695

<210> 15

<211> 2094

<212> DNA

<213> Homo sapiens

<400> 15

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ctgaacatgc acatgaatgt ccagaatggg aagtgggatt cagatccatc agggaccaaa 180

acctgcattg ataccaagga aggcatcctg cagtattgcc aagaagtcta ccctgaactg 240

<210> 16

<211> 697

<212> PRT

<213> Homo sapiens

<400> 16

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn Lys Lys
690 695

<210> 17
<211> 2094
<212> DNA
<213> Homo sapiens

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atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag 480
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<400> 18

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn Lys Lys
690 695

<210> 19

<211> 2094

<212> DNA

<213> Homo sapiens

<400> 19

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ctgaacatgc acatgaatgt ccagaatggg aagtgggatt cagatccatc agggaccaaa 180

acctgcattg ataccaagga aggcatcctg cagtattgcc aagaagtcta ccctgaactg 240

cagatcacca atgtggtaga agccaaccaa ccagtgacca tccagaactg gtgcaagcgg 300

ggccgcaagc agtgcaagac ccatccccac tttgtgattc cctaccgctg cttagttggt 360

gagtttgtaa gtgatgccct tctcgttcct gacaagtgca aattcttaca ccaggagagg 420

atggatgttt gcgaaactca tcttcactgg cacaccgtcg ccaaagagac atgcagtgag 480

aagagtacca acttgcatga ctacggcatg ttgctgccct gcggaattga caagttccga 540

ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600

gcggaggagg atgactcgga tgtctggtgg ggcggagcag acacagacta tgcagatggg 660

agtgaagaca aagtagtaga agtagcagag gaggaagaag tggctgaggt ggaagaagaa 720

gaagccgatg atgacgagga cgatgaggat ggtgatgagg tagaggaaga ggctgaggaa 780

ccctacgaag aagccacaga gagaaccacc agcattgcca ccaccaccac caccaccaca 840

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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
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Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
485 490 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
500 505 510

675 680 685

Phe Phe Glu Gln Met Gln Asn Lys Lys
690 695

<210> 21

<211> 1341

<212> DNA

<213> Homo sapiens

<400> 21

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gacgaagagc ccgaggagcc cggccggagg ggcagctttg tggagatggt ggacaacctg 180

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ctcaacatcc tggtggatac aggcagcagt aactttgcag tgggtgctgc cccccacccc 300

ttcctgcatc gctactacca gaggcagctg tccagcacat accgggacct ccggaagggt 360

gtgtatgtgc cctacaccca gggcaagtgg gaaggggagc tgggcaccga cctggtaagc 420

atcccccatg gccccaacgt cactgtgcgt gccaacattg ctgccatcac tgaatcagac 480

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cggccagtgg aagatgtggc cacgtcccaa gacgactgtt acaagtttgc catctcacag 1140

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Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly
        115                 120                 125

Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly
    130                 135                 140

Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp
145                 150                 155                 160

Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala
                165                 170                 175

Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp
            180                 185                 190

Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu His Leu
        195                 200                 205

Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val
    210                 215                 220

Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly
225                 230                 235                 240

Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile
                245                 250                 255

Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys
            260                 265                 270
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435 440 445

<210> 23

<211> 1380

<212> DNA

<213> Homo sapiens

<400> 23

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gggggcgccc ccctggggct gcggctgccc cgggagaccg acgaagagcc cgaggagccc 180

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actgtgcgtg ccaacattgc tgccatcact gaatcagaca agttcttcat caacggctcc 540

aactgggaag gcatcctggg gctggcctat gctgagattg ccaggcctga cgactccctg 600

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tttgctgtca gcgcttgcca tgtgcacgat gagttcagga cggcagcggt ggaaggccct 1320

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Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu
    130                 135                 140

Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn Val
145                 150                 155                 160

Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe
                165                 170                 175

Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu
            180                 185                 190

Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val
        195                 200                 205

Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu His Leu Cys Gly Ala
    210                 215                 220

Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser
225                 230                 235                 240

Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp
                245                 250                 255

Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg
            260                 265                 270

Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn
        275                 280                 285
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450 455

<210> 25

<211> 1302

<212> DNA

<213> Homo sapiens

<400> 25

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catgtgcacg atgagttcag gacggcagcg gtggaaggcc cttttgtcac cttggacatg 1260

gaagactgtg gctacaacat tccacagaca gatgagtcat ga 1302

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Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr
    130                 135                 140

Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu
145                 150                 155                 160

Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro
                165                 170                 175

Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser
            180                 185                 190

Leu His Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu
        195                 200                 205

Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu
    210                 215                 220

Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr
225                 230                 235                 240

Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met
                245                 250                 255

Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr
            260                 265                 270

Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser
        275                 280                 285

Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu
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<211> 1278

<212> DNA

<213> Homo sapiens

<400> 27

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aacatcctgg tggatacagg cagcagtaac tttgcagtgg gtgctgcccc ccaccccttc 240

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<210> 28

<211> 425

<212> PRT

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Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln
                165                 170                 175

Thr His Val Pro Asn Leu Phe Ser Leu His Leu Cys Gly Ala Gly Phe
            180                 185                 190

Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile
        195                 200                 205

Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr
    210                 215                 220

Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu
225                 230                 235                 240

Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp
                245                 250                 255

Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys
            260                 265                 270

Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu
        275                 280                 285

Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln
    290                 295                 300

Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu
305                 310                 315                 320
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ggcttctacg ttgtctttga tcgggcccga aaacgaattg gctttgctgt cagcgcttgc 1260

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<212> PRT

<213> Homo sapiens

<400> 30

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Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro
        195                 200                 205

Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
    210                 215                 220

Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225                 230                 235                 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
                245                 250                 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
            260                 265                 270

Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
        275                 280                 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
    290                 295                 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305                 310                 315                 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
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Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
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<211> 459

<212> PRT

<213> Homo sapiens

<400> 32

Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val

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            180                 185                 190

Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro
        195                 200                 205

Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln
    210                 215                 220

Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile
225                 230                 235                 240

Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg
                245                 250                 255

Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln
            260                 265                 270

Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val
        275                 280                 285

Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala
    290                 295                 300

Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp
305                 310                 315                 320

Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr
                325                 330                 335

Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val
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<210> 34
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<213> Homo sapiens

<400> 34
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Leu Leu Lys

<210> 35
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<212> DNA
<213> Homo sapiens

<400> 35
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<211> 36
<212> DNA
<213> Homo sapiens

<210> 40

<211> 77

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Hu-Asp2

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<210> 41

<211> 51

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Caspase 8

Cleavage Site

<400> 41

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<210> 42

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Caspase 8

<210> 46

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 6-His tag

<400> 46

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<210> 47

<211> 354

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Introduce KK motif

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csnayrsnay rh0dtgactg accactcgac caggttcact snayrctcsn asnanrmadt 180

csnayrtcna mcrstwrd0t dthharmaca hngactgacc actcgaccag gttcttdgda 240

n0bd0cda00 a0ca0rtntr ygtabwrddc mntsmmaryn rmatndcmnt smmarynrma 300

tnsks0ycmb abctrhvgrr ccr0rsmcrs twrddcmntm swrddcwrdd cmnt 354

<210> 48

<211> 462

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